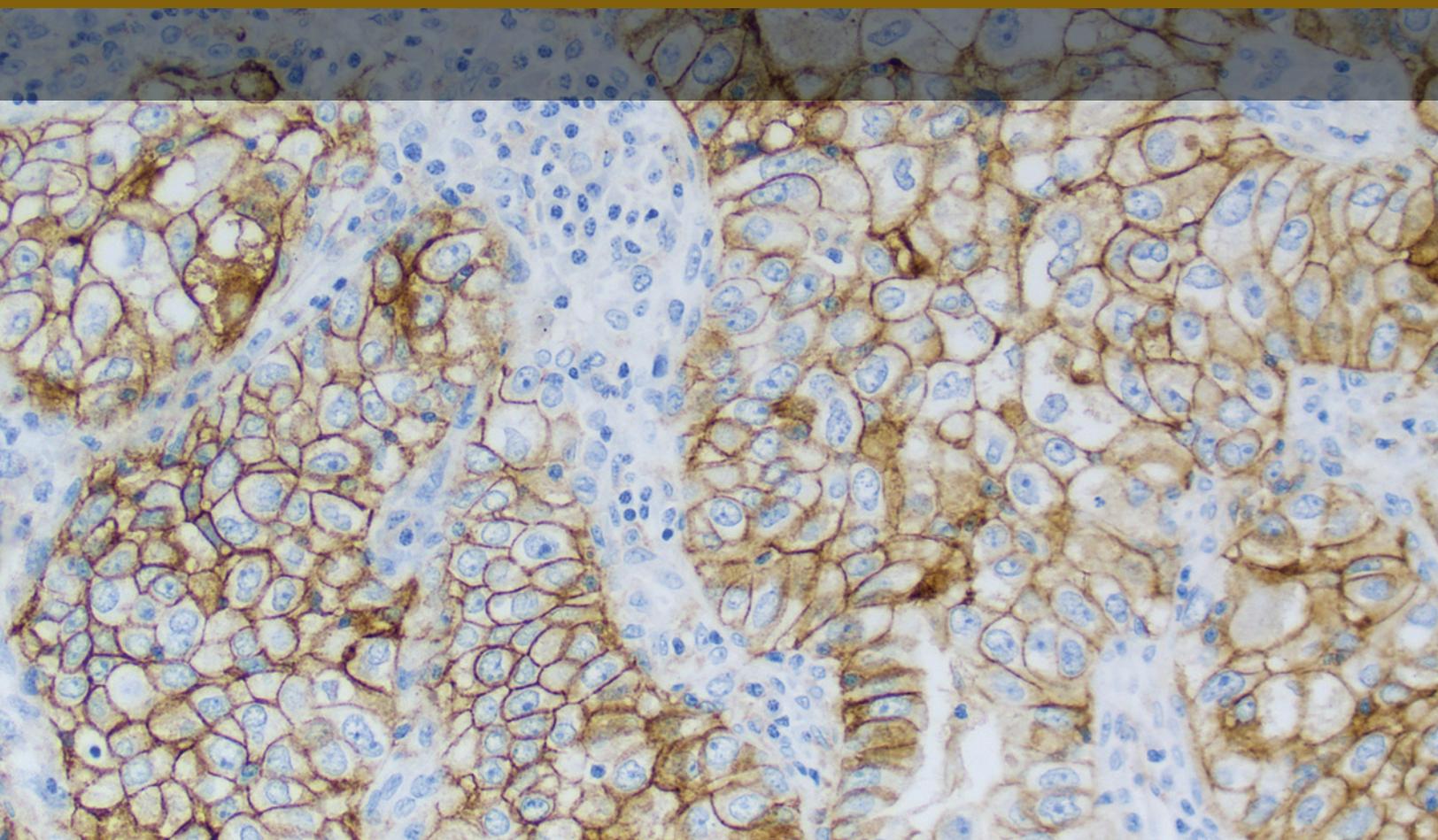


PD-L1 IHC 22C3 pharmDx Interpretation Manual – Non-small Cell Lung Cancer (NSCLC)

For in vitro diagnostic use

Rx only



Agilent

Trusted Answers

Table of Contents

Intended Use	04
Introduction	06
PD-L1 Overview	08
PD-L1 IHC 22C3 pharmDx Overview	10
Kit Configuration (SK006)	11
Technical Considerations	12
Specimen Preparation	12
Lab-supplied Control Tissue	12
Optional Additional Lab-supplied Control: Tonsil Tissue	13
Tissue Processing	13
PD-L1 IHC 22C3 pharmDx Staining Procedure	14
Technical Checklist	17
Slide Evaluation	18
General Considerations	18
Specimen Adequacy	18
Evaluating Controls	19
Slide Evaluation Flowchart	23
Evaluate Staining and Determine Tumor Proportion Score	24
Definition of Tumor Proportion Score (TPS)	24
Evaluation of PD-L1 Staining	24
Guidelines and Methods to Determine Tumor Proportion Score	25
Interpretation of TPS	26
Suggested Methods for Determining TPS	27
Identifying Patients With NSCLC for Treatment	29
Reporting Results	32
PD-L1 Staining Characteristics	33
Key Considerations in Scoring PD-L1 IHC 22C3 pharmDx Stained Specimens	33
Image Guide for Interpretation of PD-L1 IHC 22C3 pharmDx Staining in NSCLC	34
PD-L1 IHC 22C3 pharmDx TPS < 1% Case Examples	40
PD-L1 IHC 22C3 pharmDx TPS 0–10% Case Examples	44
PD-L1 IHC 22C3 pharmDx TPS 1–49% Case Examples	49
PD-L1 IHC 22C3 pharmDx TPS ≥ 50% Case Examples	53
PD-L1 IHC 22C3 pharmDx TPS 40–60% Case Examples	60
Artifacts	66
Control Cell Line (CCL) Appendix	76
Troubleshooting Guide	87
Clinical Performance Evaluation	89
References	102

Intended Use

For in vitro diagnostic use.

PD-L1 IHC 22C3 pharmDx is a qualitative immunohistochemical assay using monoclonal mouse anti-PD-L1, Clone 22C3 intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tissues using EnVision FLEX visualization system on Autostainer Link 48.

PD-L1 protein expression in NSCLC is determined by using Tumor Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity.

PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying patients for treatment with the therapies listed in Table 1.

For descriptions of the intended use in other indications, please refer to the current version of the Instructions for Use (IFU) for PD-L1 IHC 22C3 pharmDx, Code SK006.

Table 1: PD-L1 IHC 22C3 pharmDx NSCLC companion diagnostic PD-L1 expression levels and therapies

Tumor Indication	PD-L1 Expression Level	Therapy
NSCLC	TPS \geq 1%	KEYTRUDA® (pembrolizumab)*
NSCLC	TPS \geq 50%	LIBTAYO® (cemiplimab)**

KEYTRUDA is a registered trademark of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA.

LIBTAYO is a registered trademark of Regeneron Pharmaceuticals, Inc.

* See the KEYTRUDA® product label for specific clinical circumstances guiding PD-L1 testing.

** See the LIBTAYO® product label for specific clinical circumstances guiding PD-L1 testing.

Introduction

PD-L1 IHC 22C3 pharmDx is the only PD-L1 assay indicated as an aid in identifying patients with NSCLC for treatment with KEYTRUDA® (pembrolizumab) or LIBTAYO® (cemiplimab-rwlc). This Interpretation Manual is provided as a tool to help guide pathologists and laboratory personnel in achieving correct and reproducible results in assessing PD-L1 expression in formalin-fixed, paraffin-embedded non-small cell lung cancer (NSCLC) specimens. PD-L1 expression evaluation may be used to identify patients for anti-PD-1 immunotherapy.

The manual provides detailed scoring guidelines and technical information from the PD-L1 IHC 22C3 pharmDx Instructions for Use (IFU) to ensure high-quality staining and diagnostic assessment. To help familiarize you with the requirements for scoring NSCLC stains with PD-L1 IHC 22C3 pharmDx, example cases of various PD-L1 expression levels are provided as references. These example cases and in-depth recommendations for interpretation of NSCLC specimens stained with PD-L1 IHC 22C3 pharmDx can help individual labs achieve reproducible and reliable results.

PD-L1 IHC 22C3 pharmDx is considered a qualitative immunohistochemical assay. PD-L1 protein expression in NSCLC is determined by using Tumor Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity.

NSCLC tissue specimens that are tested for PD-L1 expression are scored and divided into expression levels based on a Tumor Proportion Score (TPS):

- TPS < 1%
- TPS ≥ 1% (Determines eligibility for KEYTRUDA monotherapy)
- TPS ≥ 50% (Determines eligibility for LIBTAYO monotherapy)

Note: PD-L1 expression level TPS ≥ 50% may be of interest to treating physician and includes eligibility for KEYTRUDA monotherapy.

PD-L1 expression levels are used to inform patient eligibility for treatment with KEYTRUDA or LIBTAYO. For more details on staining and interpretation, please refer to the current version of the IFU provided with PD-L1 IHC 22C3 pharmDx, Code SK006 or visit www.agilent.com.

Assay Interpretation

The clinical interpretation of any staining, or the absence of staining, must be complemented by the evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests. This product is intended for in vitro diagnostic (IVD) use.

Reporting Results

To help understand what information should be reported to the treating physician, please refer to the Reporting Results section of this manual on page 32.

Photomicrographs

The included photomicrographs are of NSCLC unless otherwise noted.

Note: Photomicrograph magnification levels may appear different from indicated in respective annotations due to adjustment of image size.

Tissue samples supplied by BioIVT (Hicksville, NY, USA).

Data and biospecimens used in this project were provided by US Biolab (Gaithersburg, MD, USA) and IOM Ricera (Viagrande, Italy) with appropriate ethics approval and through Azenta Life Sciences.

PD-L1 Overview

The PD-1/PD-L1 Pathway Controls the Immune Response in Normal Tissue

Programmed death-ligand 1 (PD-L1) is a transmembrane protein that binds to the programmed death-1 receptor (PD-1) during immune system modulation. The PD-1 receptor is typically expressed on cytotoxic T-cells and other immune cells, while the PD-L1 ligand is typically expressed on normal cells. Normal cells use the PD-1/PD-L1 interaction as a mechanism of protection against immune recognition by inhibiting the action of T-cells (Figure 1). Inactivation of cytotoxic T-cells downregulates the immune response such that the inactive T-cell is exhausted, ceases to divide, and might eventually die by programmed cell death, or apoptosis.

The Tumor Escapes Detection by Utilizing the PD-1/PD-L1 Pathway

Many tumor cells are able to upregulate the expression of PD-L1 as a mechanism to evade the body's natural immune response. Activated T-cells recognize the PD-L1 marker on the tumor cell, similar to that of a normal cell, and PD-L1 signaling renders the T-cell inactive (Figure 2). The tumor cell escapes the immune cycle, continues to avoid detection for elimination, and is able to proliferate.

Anti-PD-1 Therapy Enables the Immune Response Against Tumors

PD-1/PD-L1 interaction between tumor cells and activated T-cells (Figure 3) is a mechanistic pathway used by immunotherapeutic agents. When the tumor cell is unable to interact with the activated T-cell, the immune system remains active, helping to prevent immunosuppression.

PD-L1 IHC 22C3 pharmDx Detects PD-L1 in NSCLC Specimens

PD-L1 upregulation in NSCLC is a biomarker for response to anti-PD-1 therapy.

KEYTRUDA® (pembrolizumab)

PD-L1 IHC 22C3 pharmDx was the only companion diagnostic used in the KEYTRUDA clinical trials (KEYNOTE-010, KEYNOTE-024, and KEYNOTE-042) to evaluate the relationship between PD-L1 expression and clinical efficacy. KEYTRUDA is a humanized monoclonal PD-1-blocking antibody.

LIBTAYO® (cemiplimab-rwlc)

PD-L1 IHC 22C3 pharmDx was the only companion diagnostic used in the LIBTAYO clinical trial (Regeneron EMPOWER-Lung 1) to evaluate the relationship between PD-L1 expression and clinical efficacy. LIBTAYO is a human monoclonal PD-1 blocking antibody.

The PD-1/PD-L1 Pathway

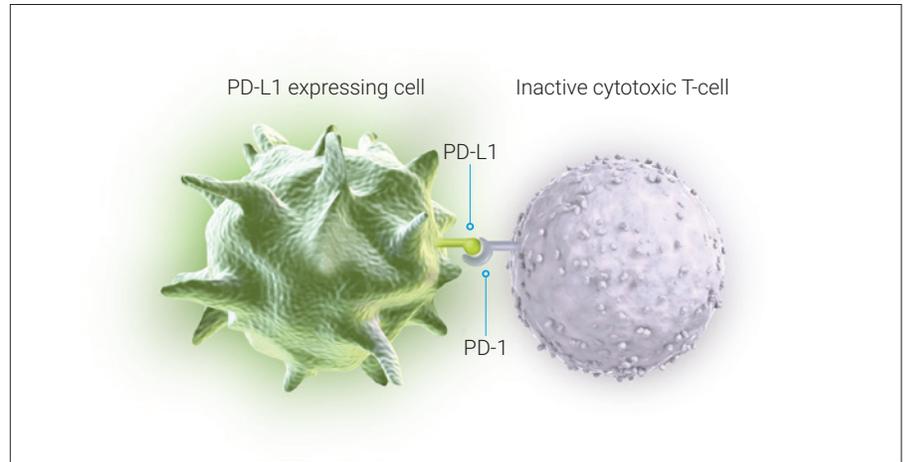


Figure 1: Inactivation of T-cells limits damage to normal tissue.

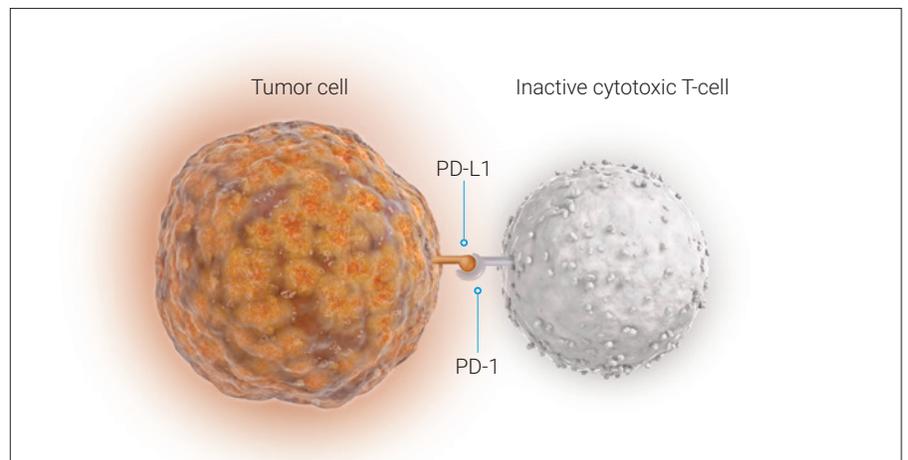


Figure 2: Inactivation of T-cells reduces tumor cell death and elimination.

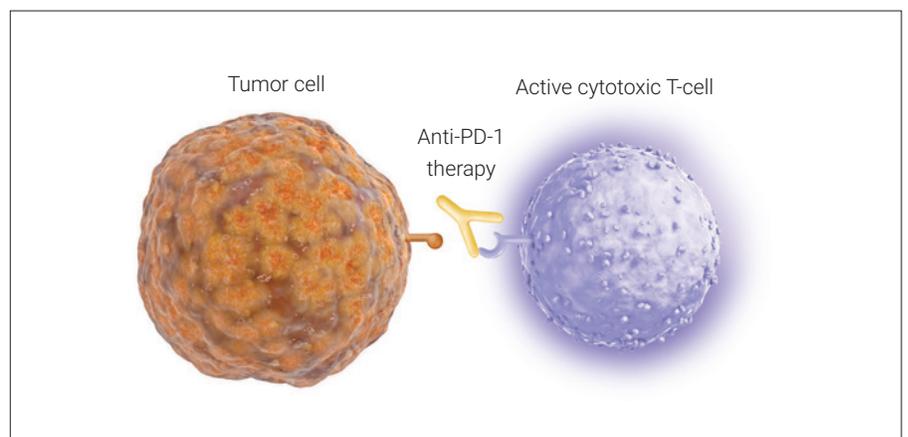


Figure 3: Blocking the PD-1/PD-L1 interaction helps to enable active T-cells and tumor cell death and elimination.

PD-L1 IHC 22C3 pharmDx Overview

What is PD-L1 IHC 22C3 pharmDx?

PD-L1 IHC 22C3 pharmDx is the only PD-L1 assay indicated as an aid in identifying patients with NSCLC for treatment with KEYTRUDA® (pembrolizumab) or LIBTAYO® (cemiplimab-rwlc). PD-L1 IHC 22C3 pharmDx is a qualitative immunohistochemical (IHC) assay intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) NSCLC tissue samples using EnVision FLEX visualization system on Autostainer Link 48.

Components of PD-L1 IHC 22C3 pharmDx

PD-L1 IHC 22C3 pharmDx contains optimized reagents to perform an IHC staining procedure using a linker and a chromogen enhancement reagent (Figure 4). Deparaffinization, rehydration, and target retrieval is performed using a 3-in-1 procedure on PT Link. Following peroxidase block, specimens are incubated with the monoclonal mouse primary antibody to PD-L1 or the Negative Control Reagent. Specimens are then incubated with a Mouse LINKER, followed by incubation with a ready-to-use Visualization Reagent consisting of secondary antibody molecules and horseradish peroxidase molecules coupled to a dextran polymer backbone.

The enzymatic conversion of the subsequently added chromogen results in precipitation of a visible reaction product at the site of the antigen. The color of the chromogenic reaction is modified by a chromogen enhancement reagent. The specimen may then be counterstained and coverslipped. Results are interpreted using a light microscope.

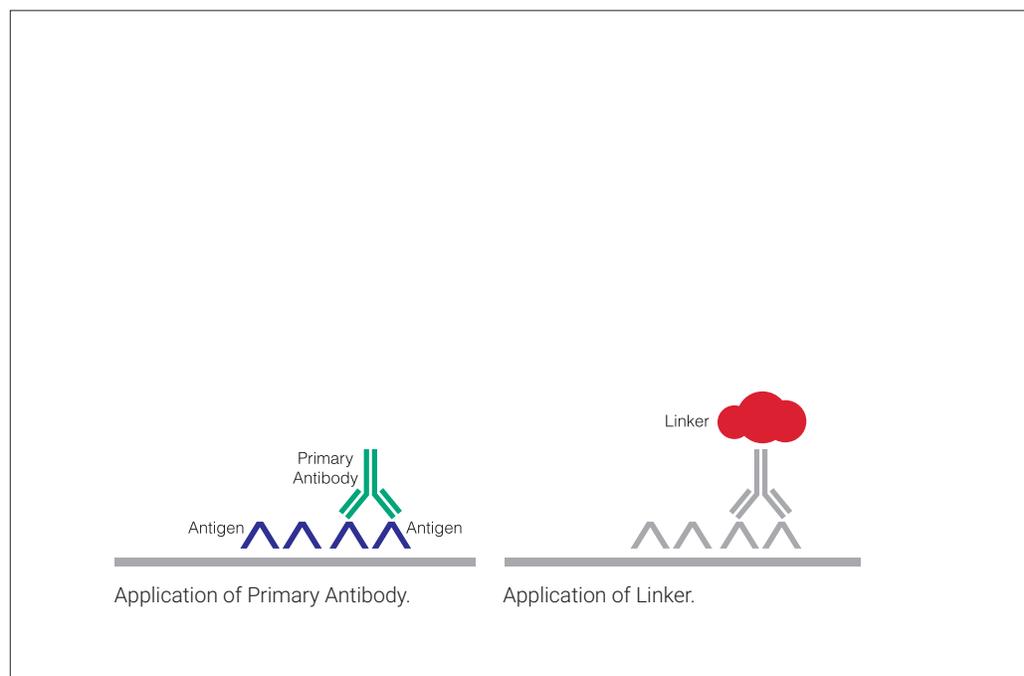


Figure 4: PD-L1 IHC 22C3 pharmDx staining procedure.

Kit Configuration (SK006)



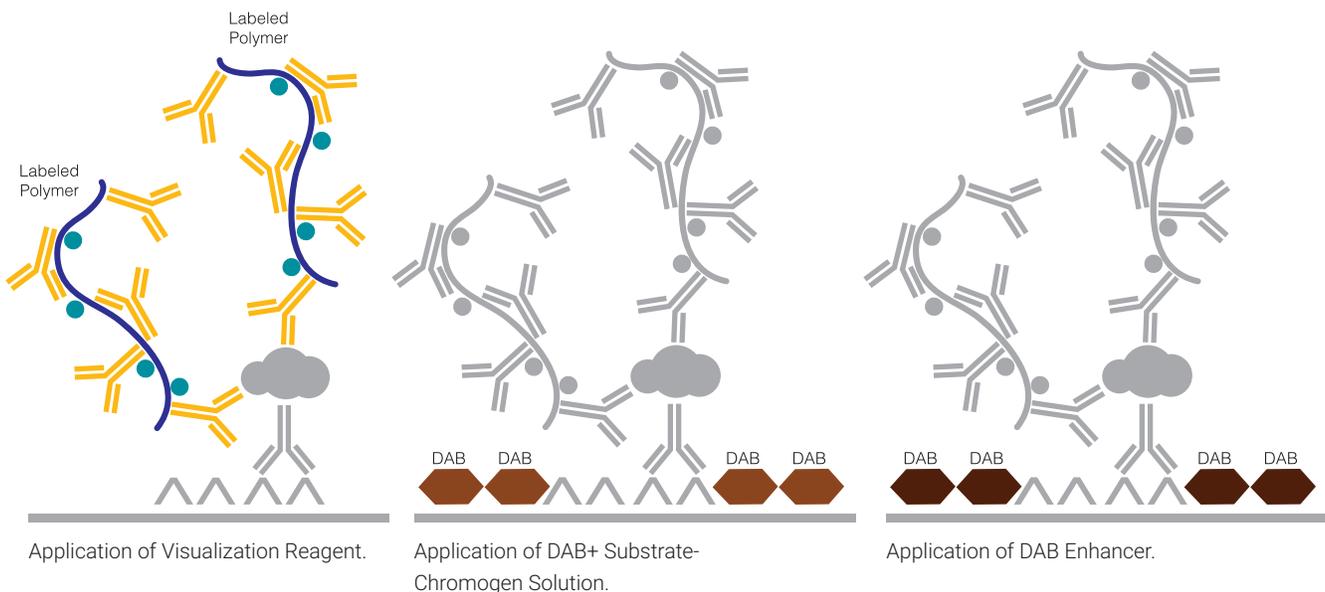
Figure 5: PD-L1 IHC 22C3 pharmDx components.

PD-L1 IHC 22C3 pharmDx (Code SK006) contains reagents to perform 50 tests in up to 15 individual runs (Figure 5):

- 1 EnVision FLEX Target Retrieval Solution, Low pH (50x)
- 2 Peroxidase-blocking Reagent
- 3 Primary Antibody: Monoclonal Mouse Anti-PD-L1, Clone 22C3
- 4 Negative Control Reagent
- 5 Mouse LINKER
- 6 Visualization Reagent-HRP
- 7 DAB+ Substrate Buffer
- 8 DAB+ Chromogen
- 9 DAB Enhancer
- 10 PD-L1 IHC 22C3 pharmDx Control Cell Line Slides*

* Dr. AF Gazdar and Dr. JD Minna at NIH are acknowledged for their contribution in developing NCI-H226 (ATCC Number: CRL-5826™)

FLEX Wash Buffer, (20x) (Code K8007), EnVision FLEX Hematoxylin (Code K8008) and wash bottle are required but not included in the kit.



Technical Considerations

Technical problems related to PD-L1 IHC 22C3 pharmDx may arise and can be attributed to two factors: specimen collection and preparation prior to performing the test, and the actual performance of the test itself. Technical problems are generally related to procedural deviations and can be controlled and minimized through training and, where necessary, clarification of the product instructions.

Specimen Preparation

Specimens must be handled to preserve the tissue for immunohistochemical staining. Determine intact tumor morphology and the presence of sufficient tumor cells for evaluation. Use standard methods of tissue processing for all specimens.

Lab-Supplied Control Tissue

Differences in processing and embedding in the user's laboratory may produce significant variability in results. Include positive and negative lab-supplied control tissue in each staining run, in addition to the PD-L1 IHC 22C3 pharmDx Control Cell Line Slide.

Refer to the 'Recommended Order of Slide Evaluation' table in the PD-L1 IHC 22C3 pharmDx IFU for additional information on appropriate control tissue. Fix, process, and embed the control tissue in the same manner. Control tissues processed differently from the patient specimen validate reagent performance only and do not verify tissue preparation.

The ideal positive control tissue provides a complete dynamic representation of weak-to-moderate staining of tumor cells. The ideal negative control tissue gives no staining on tumor cells but contains tumor-associated macrophages/immune cells which may express PD-L1 and offer an internal positive control.

Optional Additional Lab-Supplied Control: Tonsil Tissue

Tonsil stained with PD-L1 should be pre-screened to exhibit strong staining in portions of the crypt epithelium and weak-to-moderate staining of the follicular macrophages in the germinal centers. PD-L1 expression of the endothelium, fibroblasts, and the surface epithelium should be absent.

Tissue Processing

FFPE tissues have been validated for use. Block specimens into a thickness of 3 mm or 4 mm, fix in formalin and dehydrate and clear in a series of alcohols and xylene, followed by infiltration with melted paraffin. The paraffin temperature should not exceed 60 °C. Feasibility studies on NSCLC tissue samples were performed with fixation in 10% neutral buffered formalin for 12–72 hours. Fixation times of 3 hours or less should not be used for PD-L1 assessment. The use of PD-L1 IHC 22C3 pharmDx on decalcified tissues or tissues processed with other fixatives has not been validated and is not recommended.

Cut tissue specimens into sections of 4–5 µm. After sectioning, tissues should be mounted on Dako FLEX IHC Microscope Slides (Code K8020) or Superfrost Plus slides, and then placed in a 58 ± 2 °C oven for 1 hour. To preserve antigenicity, store tissue sections in the dark at 2–8 °C (preferred) or at room temperature up to 25 °C in the dark and stain within the time period specified in the IFU for each indication and temperature condition.

PD-L1 IHC 22C3 pharmDx Staining Procedure

The PD-L1 IHC 22C3 pharmDx reagents and instructions have been designed for optimal performance. Further dilution of the reagents, alteration of incubation times, temperatures, or materials may give erroneous results. All of the required steps and incubation times for staining are pre-programmed in the DakoLink software.

Reagent Storage

Store all components of PD-L1 IHC 22C3 pharmDx, including Control Cell Line Slides, in the dark at 2–8 °C when not in use.

Reagent Preparation

Equilibrate all components to room temperature (20–25 °C) prior to immunostaining. Do not use after the expiration date printed on the outside of the package.

EnVision FLEX Target Retrieval Solution, Low pH

Dilute EnVision FLEX Target Retrieval Solution, Low pH (50×) 1:50 using distilled or deionized water (reagent-quality water). One 30 mL bottle of concentrate provides 1.5 L of working solution, which is sufficient to fill one PT Link tank. Discard 1× EnVision FLEX Target Retrieval Solution, Low pH after 3 uses or 5 days after dilution.

EnVision FLEX Wash Buffer

Dilute EnVision FLEX Wash Buffer (20×) 1:20 using distilled or deionized water (reagent-quality water). Store unused 1× buffer at 2–8 °C for no more than 1 month. Discard if cloudy in appearance.

DAB+ Substrate-Chromogen Solution

Add 1 drop of DAB+ Chromogen per mL of DAB+ Substrate Buffer and mix. Prepared DAB+ Substrate-Chromogen is stable for 5 days if stored in the dark at 2–8 °C. Mix the DAB+ Substrate-Chromogen Solution thoroughly prior to use. Any precipitate developing in the solution will not affect staining quality.

- *If using an entire bottle of DAB+ Substrate Buffer, add 9 drops of DAB+ Chromogen. Although the DAB+ Substrate Buffer label states 7.2 mL, this is the usable volume and does not account for the “dead volume” of DAB+ Substrate Buffer in the bottle*
- The color of the DAB+ Chromogen may vary from clear to lavender brown. This will not affect the performance of the product. Dilute per the guidelines above. Adding excess DAB+ Chromogen to the DAB+ Substrate Buffer results in deterioration of the positive signal

Controls to Assess Staining Quality

The following quality controls should be included in each staining run:

- One PD-L1 IHC 22C3 pharmDx Control Cell Line Slide stained with the primary antibody
- Positive and negative lab-supplied control tissues stained with the primary antibody and Negative Control Reagent
- Serial section of each patient specimen stained with the Negative Control Reagent

Deparaffinization, Rehydration, and Target Retrieval

Use PT Link to perform a Deparaffinization, Rehydration, and Target Retrieval 3-in-1 procedure:

- Set Preheat and Cool to 65 °C, and set Heat to 97 °C for 20 minutes
- Fill PT Link tanks with 1.5 L per tank of 1× EnVision FLEX Target Retrieval Solution, Low pH working solution to cover the tissue sections
- Preheat the Target Retrieval Solution, Low pH to 65 °C
- Immerse Autostainer racks containing mounted, FFPE tissue sections into the preheated Target Retrieval Solution, Low pH in PT Link tank. Incubate for 20 minutes at 97 °C
- When incubation has been completed and the temperature has cooled to 65 °C, remove each Autostainer slide rack with slides from the PT Link tank and immediately place the slides into a tank (e.g., PT Link Rinse Station, Code PT109) containing room temperature 1× EnVision FLEX Wash Buffer working solution
- Leave Autostainer rack with slides in room temperature 1× EnVision FLEX Wash Buffer for 5 minutes

Staining and Counterstaining

- Place the Autostainer racks with slides one rack at a time on the Autostainer Link 48
- Prior to initiating the staining procedure on the Autostainer Link 48, 1x EnVision FLEX Wash Buffer should be manually applied to the slides using a wash bottle for prevention of tissue drying. 1x EnVision FLEX Wash Buffer should not be applied directly on the tissue section but applied sufficiently to the slide so that the tissue section is amply covered. Slides should remain wet prior to the initiation of the staining procedure. Do not allow tissue sections to dry after deparaffinization, rehydration and target retrieval (3-in-1) procedure (specimen pretreatment) or at any time during the staining procedure. Dried tissue sections may display increased nonspecific staining (including nuclear staining).
- Ensure that Autostainer slide racks are level prior to initiation of the IHC staining procedure. Level Autostainer slide racks are required for staining. Unlevel and/or warped Autostainer slide racks can result in uneven reagent distribution and improper pooling on the glass away from the specimen areas, which increases the risk for tissue drying and may lead to the appearance of nonspecific staining on the Primary Antibody and/or NCR-stained slides. Perform level testing using dry untreated slides for each slide position in all Autostainer slide racks every 3 months or whenever the Autostainer Link 48 is moved or adjusted on the counter. Discard Autostainer slide racks that fail level testing in any slide position and/or have undergone ≥ 175 PT Link pretreatment cycles
- Ensure that the Autostainer Link 48 lid is properly closed to prevent reagent evaporation during the staining procedure
- Select the PD-L1 IHC 22C3 pharmDx protocol. The instrument performs the staining and counterstaining procedures by applying the appropriate reagent, monitoring the incubation time, and rinsing slides between reagents
- Counterstain slides using EnVision FLEX Hematoxylin, Code K8008

Mounting

Use non-aqueous permanent mounting media. To minimize fading, store slides in the dark at room temperature (20–25 °C).

Technical Checklist

Use the checklist below to ensure correct usage of PD-L1 IHC 22C3 pharmDx:

Customer Name/Institution _____

Name and Title _____

Autostainer Link 48 Serial Number _____ Software Version _____

	Yes	No
Regular preventive maintenance is performed on the Autostainer Link 48 and PT Link?	<input type="checkbox"/>	<input type="checkbox"/>
PD-L1 IHC 22C3 pharmDx is used before the expiration date printed on the outside of the box?	<input type="checkbox"/>	<input type="checkbox"/>
All PD-L1 IHC 22C3 pharmDx components, including Control Cell Line Slides, are stored in the dark at 2–8 °C?	<input type="checkbox"/>	<input type="checkbox"/>
All PD-L1 IHC 22C3 pharmDx components, including Control Cell Line Slides, are equilibrated to room temperature (20–25 °C) prior to immunostaining?	<input type="checkbox"/>	<input type="checkbox"/>
Appropriate positive and negative control tissues are identified?	<input type="checkbox"/>	<input type="checkbox"/>
Tissues are fixed in neutral buffered formalin?	<input type="checkbox"/>	<input type="checkbox"/>
Tissues are infiltrated with melted paraffin, at or below 60 °C?	<input type="checkbox"/>	<input type="checkbox"/>
Tissue sections of 4–5 µm are mounted on Dako FLEX IHC Microscope Slides or Superfrost Plus slides?	<input type="checkbox"/>	<input type="checkbox"/>
Specimens are oven-dried at 58 ± 2 °C for 1 hour?	<input type="checkbox"/>	<input type="checkbox"/>
Specimen tissue sections are stained within the indication- and storage condition-specific time period from the date of sectioning given in the IFU?	<input type="checkbox"/>	<input type="checkbox"/>
EnVision FLEX Target Retrieval Solution, Low pH is prepared properly? pH of 1× Target Retrieval Solution must be 6.1 ± 0.2.	<input type="checkbox"/>	<input type="checkbox"/>
EnVision FLEX Wash Buffer is prepared properly?	<input type="checkbox"/>	<input type="checkbox"/>
DAB+ Substrate-Chromogen Solution is prepared properly?	<input type="checkbox"/>	<input type="checkbox"/>
Slides are counterstained with EnVision FLEX Hematoxylin?	<input type="checkbox"/>	<input type="checkbox"/>
The Deparaffinization, Rehydration, and Target Retrieval 3-in-1 procedure is followed using PT Link?	<input type="checkbox"/>	<input type="checkbox"/>
Are the slide racks level?	<input type="checkbox"/>	<input type="checkbox"/>
EnVision FLEX Wash Buffer is manually applied to slides using a wash bottle and slides remain wet with buffer while loading and prior to initiating run on Autostainer Link 48?	<input type="checkbox"/>	<input type="checkbox"/>
The PD-L1 IHC 22C3 pharmDx protocol is selected on Autostainer Link 48?	<input type="checkbox"/>	<input type="checkbox"/>
Autostainer Link 48 lid is properly closed during staining procedure?	<input type="checkbox"/>	<input type="checkbox"/>
Do you have all the necessary equipment to perform the PD-L1 IHC 22C3 pharmDx according to protocol? If not, specify what is missing in comments below.	<input type="checkbox"/>	<input type="checkbox"/>

Additional observations or comments:

Slide Evaluation

General Considerations

PD-L1 IHC 22C3 pharmDx evaluation should be performed by a qualified pathologist using a light microscope. Details of the PD-L1 IHC 22C3 pharmDx interpretation guidelines are reviewed on page 26. Before examining the patient specimen for PD-L1 staining, it is important to examine the controls to assess staining quality.

PD-L1 expression is best assessed by requesting 3 serial tissue sections (H&E, PD-L1 stain, and NCR stain) so that if the H&E is first assessed and is acceptable, the 2 remaining serial sections are likely to retain the same favorable tissue quality.

Each PD-L1 IHC 22C3 pharmDx is configured with Control Cell Line Slides that should be included in each IHC run. Guidelines on interpreting the Control Cell Line Slide are reviewed to the right. Lab-supplied control tissue slides should also be assessed with every IHC run.

Specimen Adequacy

Confirm the Presence of at Least 100 Viable Tumor Cells

A hematoxylin and eosin (H&E) stain of the tissue specimen is evaluated first to assess tissue histology and preservation quality. PD-L1 IHC 22C3 pharmDx and the H&E staining should be performed on serial sections from the same paraffin block of the specimen. Tissue specimens should be intact, well preserved, and should confirm tumor indication.

A minimum of 100 viable tumor cells must be present in the PD-L1 stained slide for the specimen to be considered adequate for PD-L1 evaluation.

Instructions for Patient Specimens With Less Than 100 Viable Tumor Cells

Tissue from a deeper level of the block, or potentially another block, could have a sufficient number of viable tumor cells for PD-L1 IHC 22C3 pharmDx testing.

Evaluating Controls

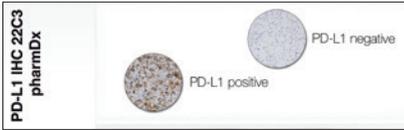


Figure 6: Each Control Cell Line Slide contains sections of cell pellets with positive and negative PD-L1 expression.

PD-L1 IHC 22C3 pharmDx Control Cell Line Slide

Examine the PD-L1 IHC 22C3 pharmDx Control Cell Line Slide to determine that reagents are functioning properly. Each slide contains sections of cell pellets with positive and negative PD-L1 expression (Figure 6). Assess the percentage of positive cells, staining intensity, and nonspecific staining in both cell pellets. If any staining of the Control Cell Line Slide is not satisfactory, all results with the patient specimens should be considered invalid. Do not use the Control Cell Line Slide as an aid in interpretation of patient results.

Evaluate the overall staining intensity using the following guide:

0	Negative
1+	Weak intensity
2+	Moderate intensity
3+	Strong intensity

Positive Control Cell Pellet

The following staining is acceptable for the PD-L1 positive cell pellet (Figure 7):

- Cell membrane staining of $\geq 70\%$ of cells
- $\geq 2+$ average staining intensity of cells with membrane staining
- Nonspecific staining $< 1+$ intensity

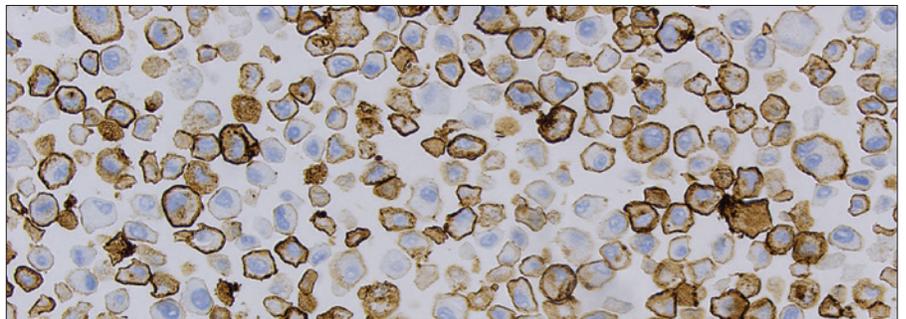


Figure 7: Positive cell pellet with acceptable staining of PD-L1 IHC 22C3 pharmDx Control Cell Line Slide (20x magnification).

Negative Control Cell Pellet

For the PD-L1 negative cell pellet, the following staining is acceptable (Figure 8):

- No cells with membrane staining*
- Nonspecific staining < 1+ intensity*

*Note that staining of a few cells in the MCF-7 cell pellet may occasionally be observed. The following acceptance criteria are applicable: the presence of ≤ 10 total cells with distinct cell membrane staining and/or nonspecific staining with $\geq 1+$ intensity within the boundaries of the MCF-7 cell pellet are acceptable.

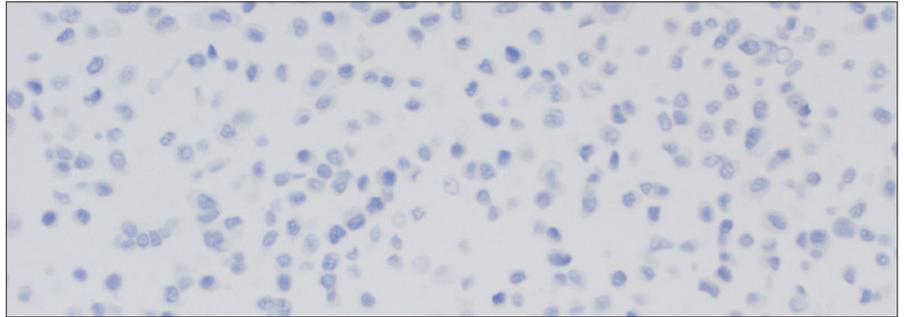


Figure 8: Negative cell pellet with no staining of PD-L1 IHC 22C3 pharmDx Control Cell Line Slide (20x magnification).

See the Control Cell Line (CCL) Appendix on page 76 for images of passing, borderline, and failing control cell line staining.

Positive and Negative Lab-supplied Control Tissue

Examine the positive lab-supplied control tissue to determine that the tissues are correctly prepared and reagents are functioning properly. The ideal positive control tissue provides a complete dynamic representation of weak-to-moderate tumor cell membrane staining (Figure 9). If staining of positive lab-supplied control tissue is not satisfactory, all results with the patient specimen should be considered invalid.

- Requirements for slide stained with PD-L1: Presence of brown cell membrane staining should be observed. Nonspecific staining, including nuclear staining, should be $\leq 1+$
- Requirements for slide stained with Negative Control Reagent: No membrane staining. Nonspecific staining, including nuclear staining, should be $\leq 1+$

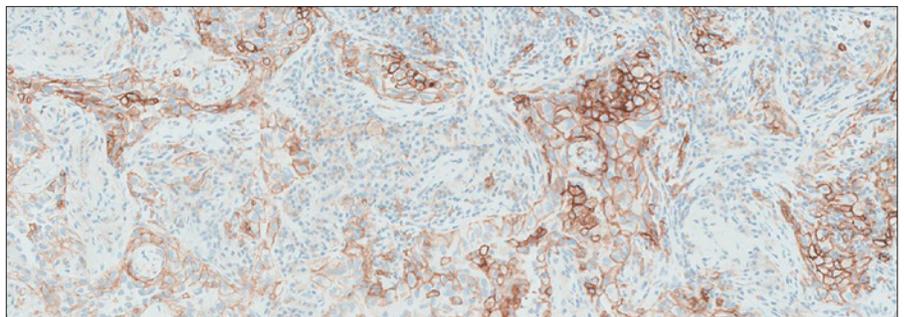


Figure 9: Ideal positive lab-supplied control tissue stained with PD-L1 primary antibody (10x magnification).

The ideal negative control tissue demonstrates no staining on tumor cells but contains tumor-associated macrophages/immune cells that express PD-L1 and offer an internal positive control (Figure 10). Examine the negative lab-supplied control tissue to determine the expected staining. The variety of different cell types present in most tissue sections offers internal negative control sites; this should be verified by the user.

If unwanted staining occurs in the lab-supplied control tissues, results with the patient specimen should be considered invalid.

- Requirements for slide stained with PD-L1: No membrane staining in tumor cells. Nonspecific staining, including nuclear staining, should be $\leq 1+$
- Requirements for slide stained with Negative Control Reagent: No membrane staining. Nonspecific staining, including nuclear staining, should be $\leq 1+$

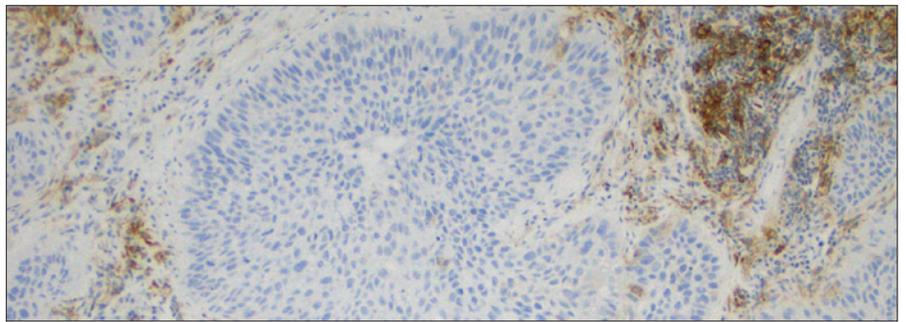


Figure 10: Ideal negative lab-supplied control tissue stained with PD-L1 primary antibody demonstrating lack of staining tumor cells and PD-L1 staining immune cells (10x magnification).

Optional Control Tissue

In addition to the Control Cell Line Slide and lab-supplied control tissues, FFPE tonsil may also be used as an optional control specimen. Tonsil stained with PD-L1 should exhibit strong membrane staining in portions of the crypt epithelium and weak-to-moderate membrane staining of the follicular macrophages in the germinal centers (Figure 11).

PD-L1 expression of the endothelium, fibroblasts, and the surface epithelium should be absent.

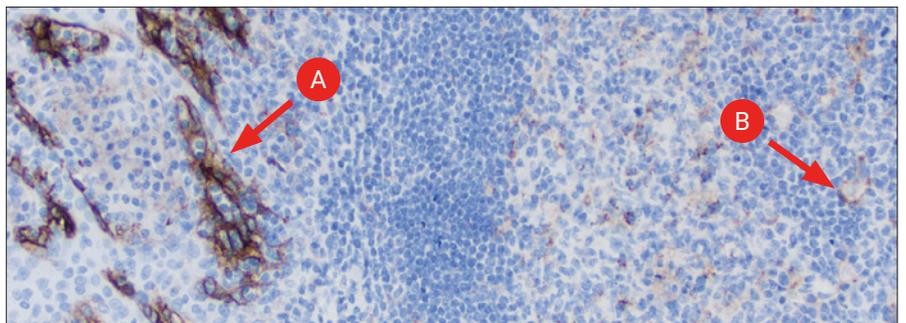


Figure 11: Tonsil stained with PD-L1 primary antibody exhibiting strong membrane staining in portions of the crypt epithelium (A) and weak-to-moderate membrane staining of follicular macrophages in the germinal centers (B) (10x magnification).

Do not use lab-supplied control tissue as an aid in interpretation of patient results.

Negative Control Reagent (NCR)

Examine the slides stained with the NCR to identify nonspecific staining that may interfere with PD-L1 staining interpretation, making the specimen non-evaluable. Satisfactory performance is indicated by the absence of staining (Figure 12).

Examine the patient specimens stained with the NCR to determine if there is any nonspecific staining that may interfere with interpreting the PD-L1 stained slide. Nonspecific staining, including nuclear staining, should be $\leq 1+$.

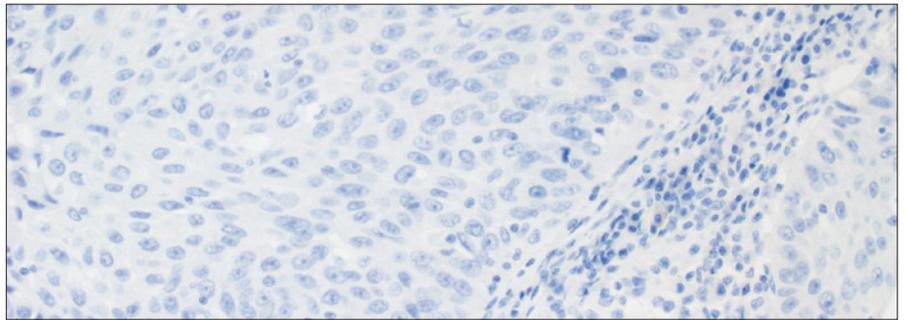


Figure 12: NSCLC tissue specimen stained with NCR (20× magnification).

NCR-stained slides indicate nonspecific staining and allow for better interpretation of patient specimens stained with the primary antibody.

Slide Evaluation Flowchart

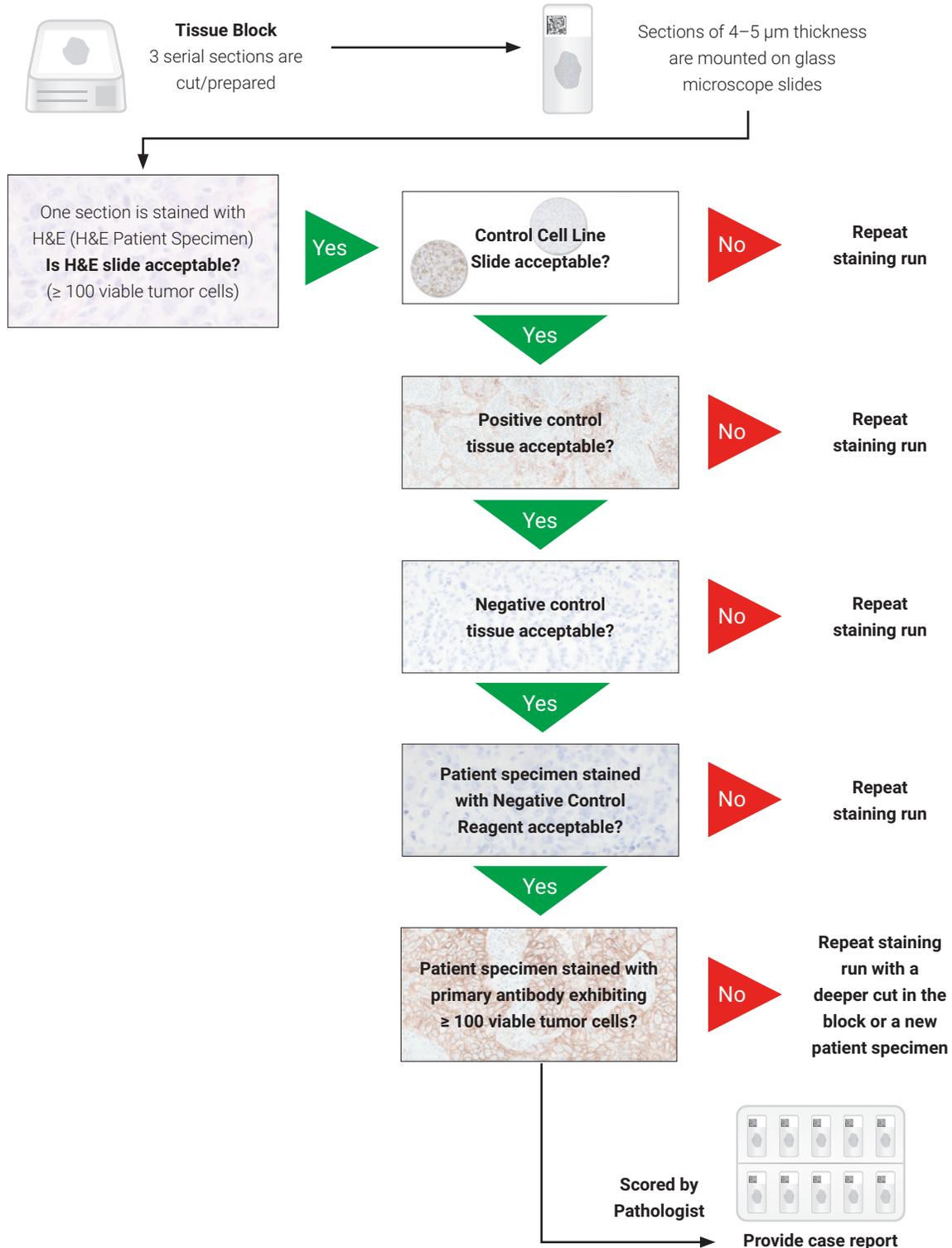


Figure 13: Recommended order of slide evaluation.

Evaluate Staining and Determine Tumor Proportion Score

Definition of Tumor Proportion Score (TPS)

The Tumor Proportion Score is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity ($\geq 1+$) relative to all viable tumor cells present in the sample.

TPS is defined accordingly:

$$\text{TPS (\%)} = \frac{\text{\# PD-L1 staining cells (tumor cells)}}{\text{Total \# of viable tumor cells}} \times 100$$

Table 2: TPS Numerator Inclusion/Exclusion Criteria for NSCLC

Tissue Elements	Included in TPS Scoring for NSCLC	Excluded from TPS Scoring for NSCLC
Tumor Cells	Convincing partial or complete cell membrane staining (at any intensity) of viable tumor cells	Exclude any cytoplasmic staining
Immune Cells	Not included	Exclude any staining of immune cells, such as: <ul style="list-style-type: none"> - Mononuclear inflammatory cells (large lymphocytes, monocytes, pulmonary macrophages) - Plasma cells - Neutrophils
Other Cells	Not included	Exclude any staining of: <ul style="list-style-type: none"> - Normal cells adjacent to tumor cells - Stromal cells (fibroblasts) - Necrotic cells and/or cellular debris - Anthracotic pigment

Evaluation of PD-L1 Staining

Score partial or complete cell membrane staining ($\geq 1+$) of tumor cells that is perceived distinct from cytoplasmic staining.

Score only viable tumor cells. Exclude any staining of immune cells, such as mononuclear inflammatory cells (large lymphocytes, monocytes, pulmonary macrophages), plasma cells, and neutrophils. Exclude any staining of normal cells adjacent to tumor cells, stromal cells (fibroblasts), necrotic cells and/or cellular debris, as well as anthracotic pigment.

Guidelines and Methods to Determine Tumor Proportion Score

- At low magnification, examine all well-preserved tumor areas. Evaluate overall areas of PD-L1 staining tumor cells, keeping in mind that partial membrane staining or $\geq 1+$ membrane staining may be difficult to see at low magnification. Ensure there are at least 100 viable tumor cells in the sample
 - At higher magnifications, including 10 \times , 20 \times , and 40 \times , observe all tumor areas with and without cell membrane staining
 - At this stage of working with multiple magnifications, primary analysis involves:
 - Distinguishing tumor cells from tumor-associated immune cells
 - Determining PD-L1 staining and nonstaining tumor areas
 - Determining partial and complete membrane staining ($\geq 1+$) of tumor cells
 - Calculate the Tumor Proportion Score by evaluating the percentage of PD-L1 staining tumor cells relative to all viable tumor cells present in the specimen
- Note:** Carefully consider the overall tumor area without any perceptible and convincing cell membrane staining

Make Sure to *Exclude* Immune Cells and Necrotic Tissue From Scoring

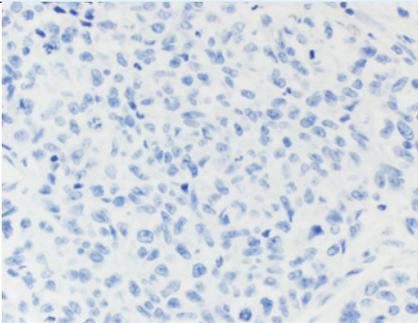
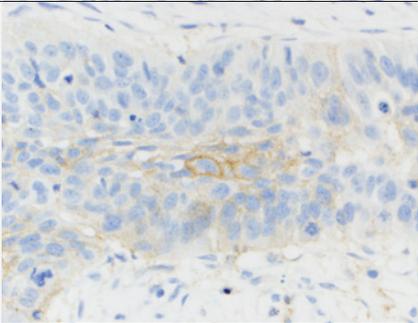
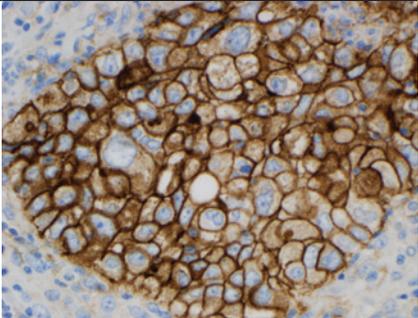
The following considerations can help distinguish tumor cells from immune cells:

- Immune cells may have smaller nuclei than tumor cells
- Macrophages may contain pigmented particles in their cytoplasm
- Macrophages may have a scattered distribution. Pulmonary macrophages are present in the alveolar space

Interpretation of TPS

The TPS determines the PD-L1 expression levels of the specimen. See the table below for scoring guideline examples.

Table 3: TPS and PD-L1 Expression Levels

TPS	Expression Level	Image (20× magnification)
< 1%	TPS is less than 1%	
≥ 1%	TPS is greater than or equal to 1%	
≥ 50%	TPS is greater than or equal to 50%	

Suggested Methods for Determining TPS

Agilent recommends that scoring be performed within the context of the pathologist’s past experience and best judgment in interpreting IHC stains. We offer two different examples of techniques that may be used when determining the respective Tumor Proportion Score of various staining patterns.

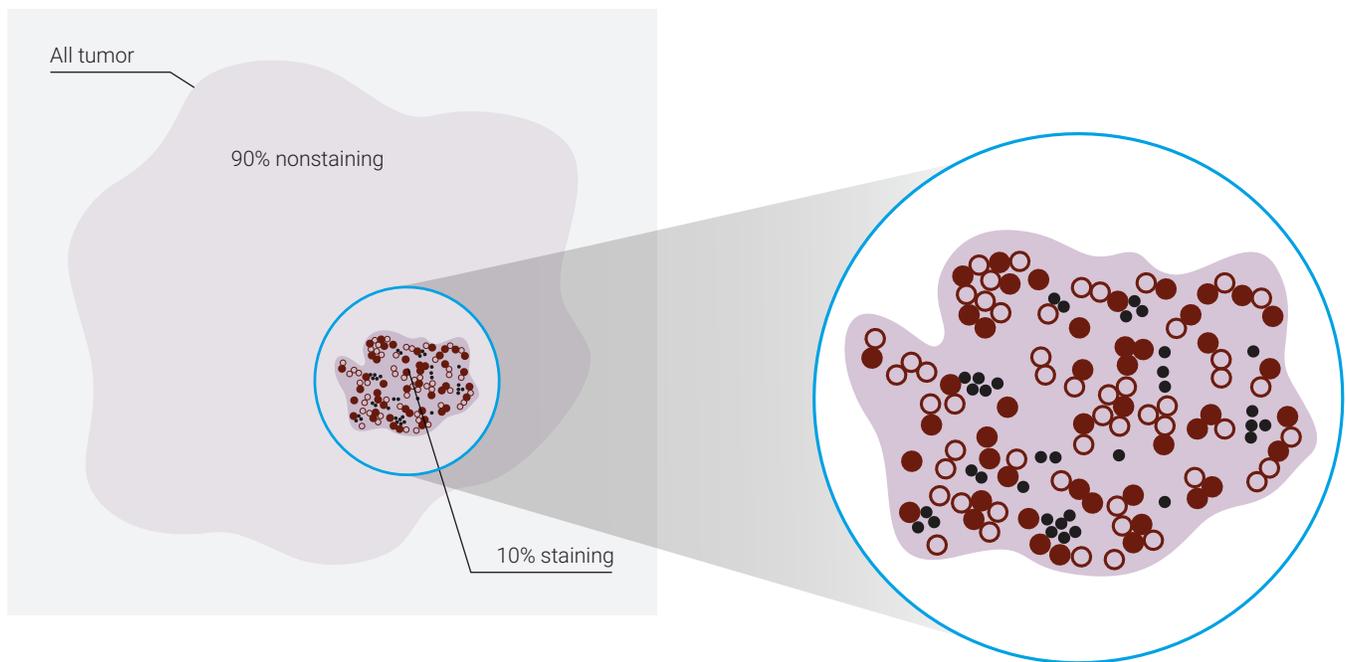
Example 1: Calculation of Tumor Proportion Score Based on a Small PD-L1 Staining Area

At lower magnification: Evaluate the tumor area for any perceptible and convincing $\geq 1+$ cell membrane staining.

Assessment: 10% of area shows staining; 90% of area shows no staining

At higher magnification: Evaluate the area of staining to estimate the percentage of PD-L1 staining tumor cells.

Assessment: 50% of tumor cells are PD-L1 staining



Calculate Tumor Proportion Score: Determine the overall percentage of PD-L1 staining tumor cells for the entire tumor area.

Assessment: Tumor Proportion Score (TPS):

$$10\% \times 50\% = 5\%$$

Clinical Interpretation: $TPS \geq 1\%$

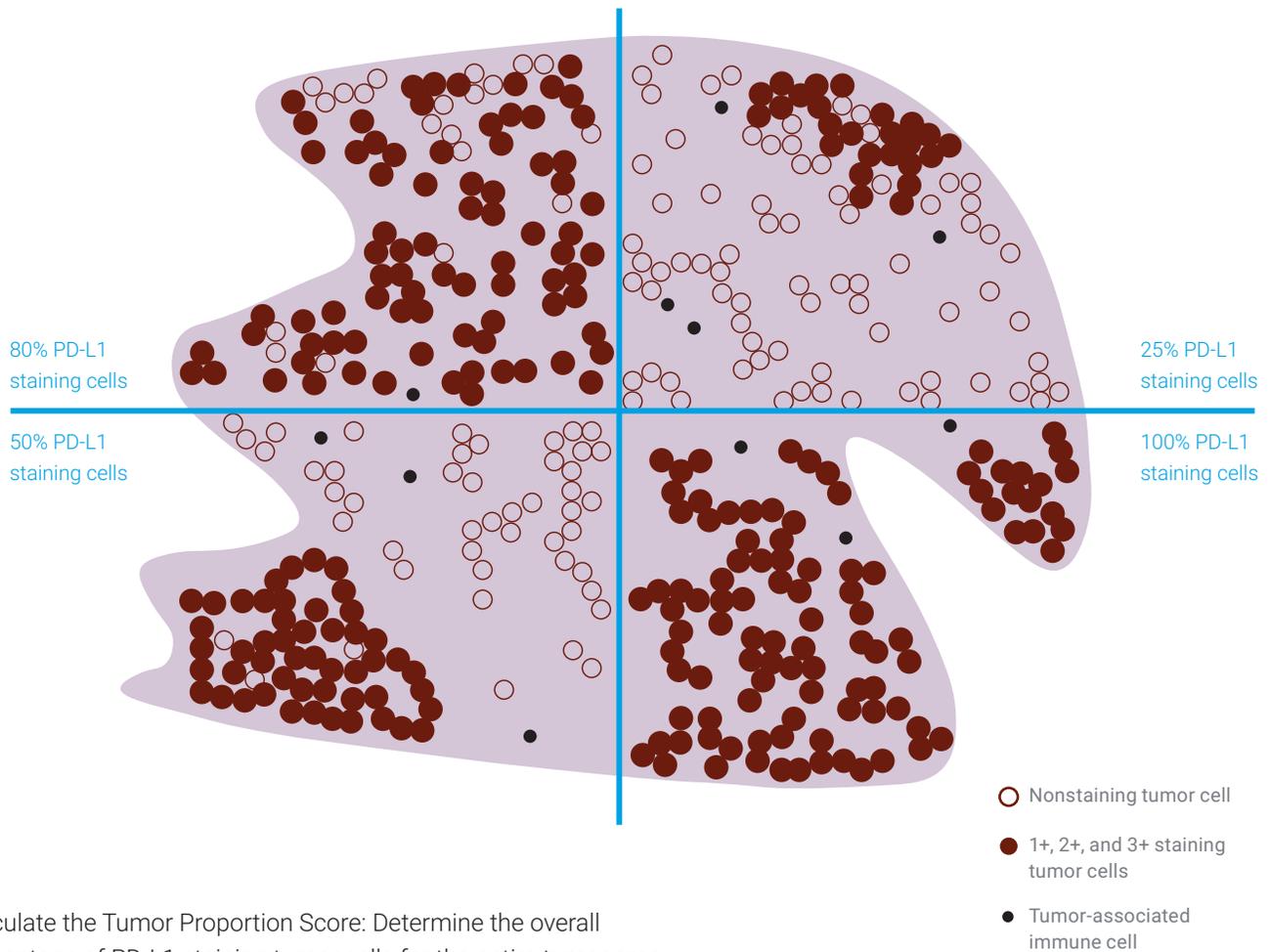
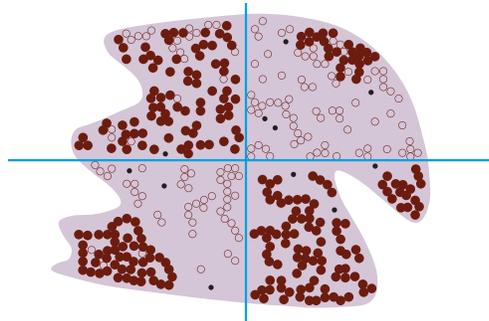
Figure 14: Example of tumor with small PD-L1 staining area.

Example 2: Calculation of Tumor Proportion Score Based on a Heterogeneous PD-L1 Staining Area

At lower magnification: Visually divide the tumor area into sections.

At higher magnification: Observe tumor areas with cell membrane staining for percentage of PD-L1 staining cells in each section.

Assessment: Percentage of PD-L1 staining cells in each of the four respective sections: 80%, 25%, 50%, 100%



Calculate the Tumor Proportion Score: Determine the overall percentage of PD-L1 staining tumor cells for the entire tumor area.

Assessment: Tumor Proportion Score (TPS):

$$(80\% + 25\% + 50\% + 100\%) / 4 \approx 60\%$$

Clinical Interpretation: $TPS \geq 50\%$

Figure 15: Example with heterogeneous PD-L1 staining area.

Identifying Patients With NSCLC for Treatment

KEYTRUDA® (pembrolizumab)

PD-L1 IHC 22C3 pharmDx is the only PD-L1 assay indicated as an aid in identifying patients with NSCLC for treatment with KEYTRUDA.

Clinical validation of PD-L1 IHC 22C3 pharmDx in previously untreated patients with NSCLC (first-line)

The clinical validity of PD-L1 IHC 22C3 pharmDx in identifying PD-L1 expression (TPS \geq 1%) in previously untreated patients with NSCLC is based on the KEYTRUDA KEYNOTE-042 study, sponsored by Merck Sharp & Dohme LLC. Specimens from previously untreated patients with NSCLC were tested for PD-L1 expression using PD-L1 IHC 22C3 pharmDx. Only patients with TPS \geq 1% were included in the KEYNOTE-042 study. Efficacy of KEYTRUDA treatment in patients selected by PD-L1 IHC 22C3 pharmDx is presented in the Clinical Performance Evaluation section on pages 85–93.

Table 4: Patient Characteristics in Patients with NSCLC Screened for KEYNOTE-042 (N=1274)*

PD-L1 Expression Level	TPS 1–49%	TPS \geq 50%
% of Patients	53%	47%

* Percentage calculation based on patients whose tumors expressed PD-L1 (TPS \geq 1%). Patients whose tumors did not express PD-L1 were not enrolled

PD-L1 IHC 22C3 pharmDx Testing Scheme

Use the following flowchart to help you understand which patients are indicated for treatment with KEYTRUDA® (pembrolizumab) based on their TPS.

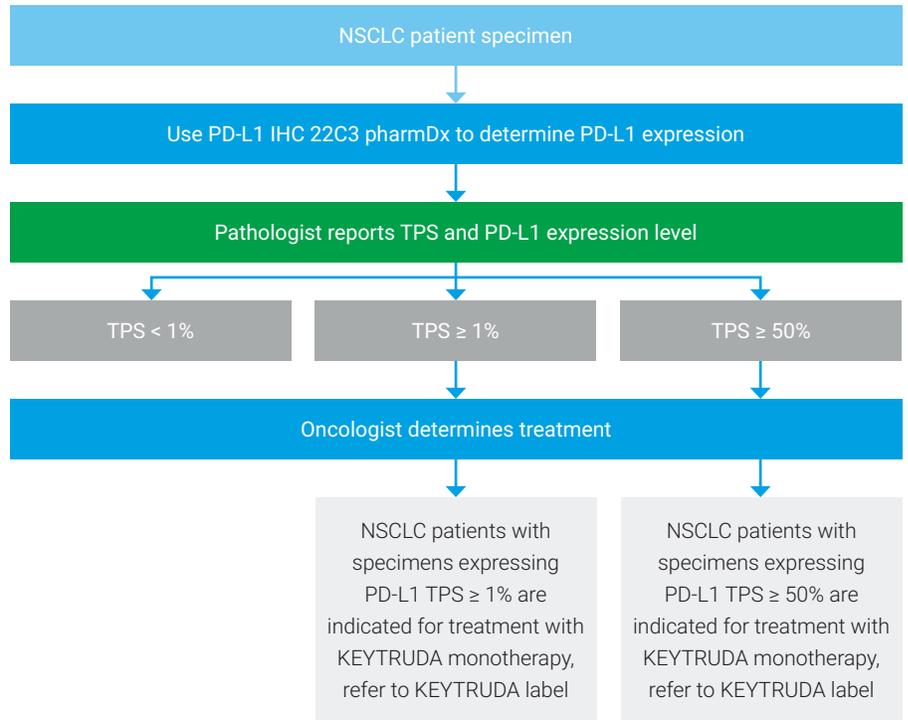


Figure 16: Testing scheme for PD-L1 IHC 22C3 pharmDx.

LIBTAYO® (cemiplimab-rwlc)

PD-L1 IHC 22C3 pharmDx is the only PD-L1 assay indicated as an aid in identifying patients with NSCLC for treatment with LIBTAYO.

Clinical validation of PD-L1 IHC 22C3 pharmDx in patients with NSCLC (first-line)

The clinical validity of PD-L1 IHC 22C3 pharmDx in identifying PD-L1 expression (TPS \geq 50%) in patients with NSCLC is based on EMPOWER-Lung 1, sponsored by Regeneron Pharmaceuticals, Inc. Specimens from patients with NSCLC were tested for PD-L1 expression using PD-L1 IHC 22C3 pharmDx. Efficacy of LIBTAYO treatment in patients who had PD-L1 expression of TPS \geq 50% by PD-L1 IHC 22C3 pharmDx is presented in the Clinical Performance Evaluation section on pages 94–97.

PD-L1 IHC 22C3 pharmDx Testing Scheme

Use the following flowchart to help you understand which patients are indicated for treatment with LIBTAYO based on their TPS.

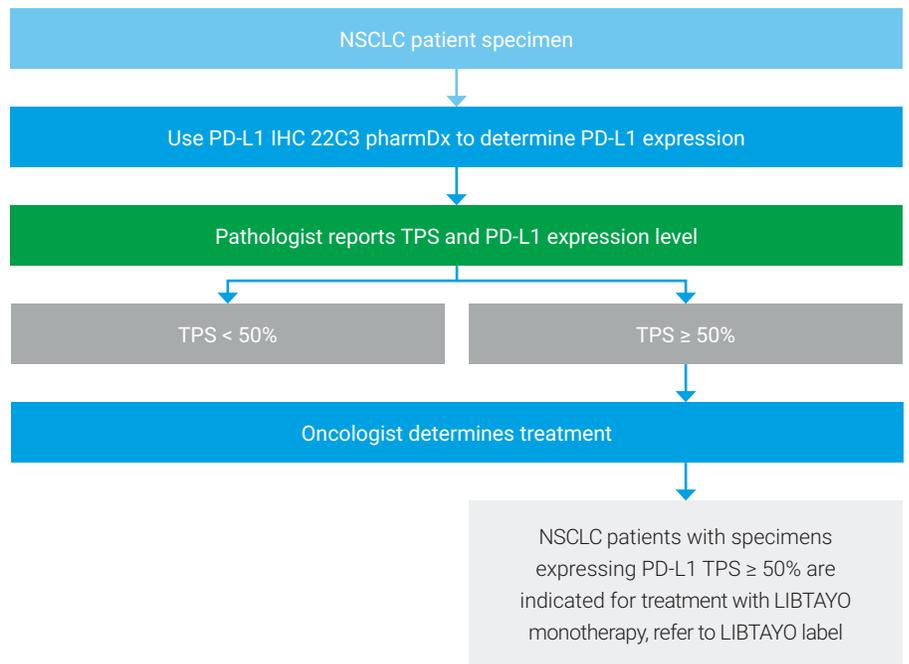


Figure 17: Testing scheme for PD-L1 IHC 22C3 pharmDx.

Reporting Results

Suggested information to include when reporting results with PD-L1 IHC 22C3 pharmDx.

PD-L1 IHC 22C3 pharmDx Summary of Sample Tested

Date of Run: _____

PD-L1 IHC 22C3 pharmDx Lot: _____

Staining Run Log ID: _____

Specimen ID: _____

Patient Identifiers: _____

Type of Service: IHC Stain With Manual Interpretation

Other: _____

PD-L1 Included in Non-small Cell Lung Cancer Comprehensive Panel: Yes: No:

Type of Tissue: Squamous Cell: Non-squamous Cell:

PD-L1 Testing Results

Control Cell Line Slide Results: Pass: Fail:

Adequate Tumor Cells Present (≥ 100 cells):

PD-L1 IHC 22C3 pharmDx Result to Treating Physician

Tumor Proportion Score (TPS): _____

TPS < 1%: TPS \geq 1%: TPS \geq 50%:

Note:

- PD-L1 expression level TPS \geq 1% determines eligibility for KEYTRUDA® (pembrolizumab). PD-L1 expression level TPS \geq 50% may be of interest to treating physician and includes eligibility for KEYTRUDA monotherapy
- PD-L1 expression level TPS \geq 50% determines eligibility for LIBTAYO® (cemiplimab-rwlc) monotherapy

PD-L1 Staining Characteristics

Key Considerations in Scoring PD-L1 IHC 22C3 pharmDx Stained Specimens

To successfully score PD-L1 IHC 22C3 pharmDx stained specimens, it is critical that:

- A minimum of 100 viable tumor cells are present for evaluation
- The appropriate cells are evaluated—only viable tumor cells should be scored
- The proper cellular localization is identified—only membrane staining of tumor cells should be evaluated
- The staining is properly interpreted

The pathologist's experience and judgment are important in the evaluation of PD-L1 staining. For evaluation of the immunohistochemical staining and scoring, objectives of 10x, 20x, and 40x magnifications are appropriate.

However, below are several staining characteristic patterns that should be considered in the Tumor Proportion Score (TPS) calculation:

- Membrane staining of tumor cells at all intensities (1–3+) should be included
- Partial and/or complete membrane staining should be included
- Any perceptible and convincing membrane staining should be included
- Cytoplasmic staining **should not** be included
- Tumor-associated immune cells such as infiltrating lymphocytes or macrophages **should not** be included
- Granular staining must demonstrate a perceptible and convincing membrane pattern to be included

The following pages provide guidance on various staining characteristics.

Image Guide for Interpretation of PD-L1 IHC 22C3 pharmDx Staining in NSCLC

Perceptible and Convincing Membrane Staining

Scoring should include any perceptible and convincing membrane staining at any intensity ($\geq 1+$) and at any magnification. Review at higher magnification may be needed to confirm perceptible and convincing membrane staining.

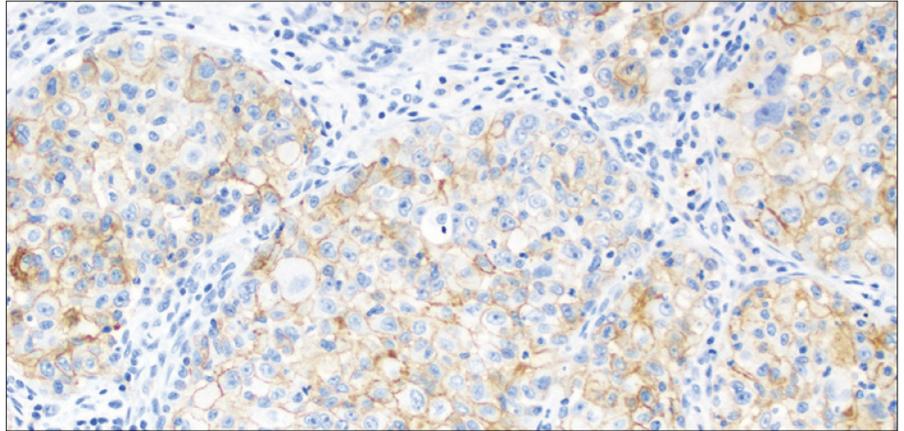


Figure 18a: NSCLC specimen stained with PD-L1 primary antibody exhibiting weak membrane staining of tumor cells (10 \times magnification).

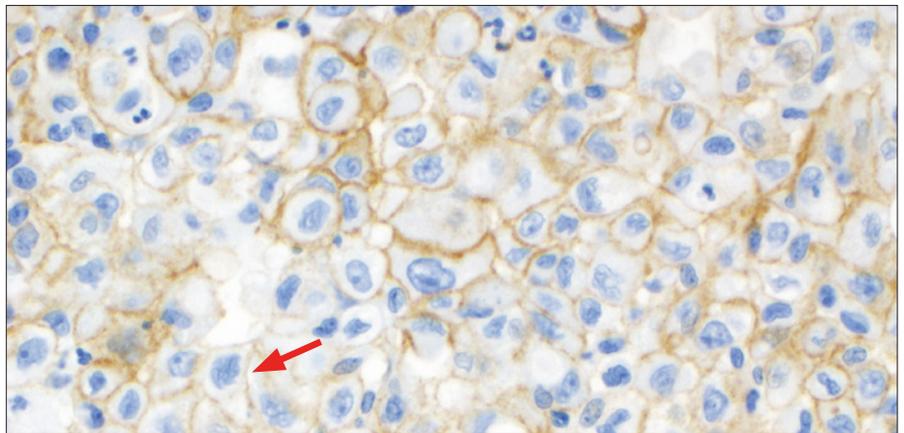


Figure 18b: NSCLC specimen stained with PD-L1 primary antibody exhibiting weak but perceptible and convincing membrane staining of tumor cells (arrow) (40 \times magnification).

Key Point

Any perceptible and convincing membrane staining of tumor cells ($\geq 1+$) should be included in the TPS

Weak Acceptable Membrane Staining

Scoring of tumor cells should include any perceptible and convincing membrane staining, including weak intensity of 1+.

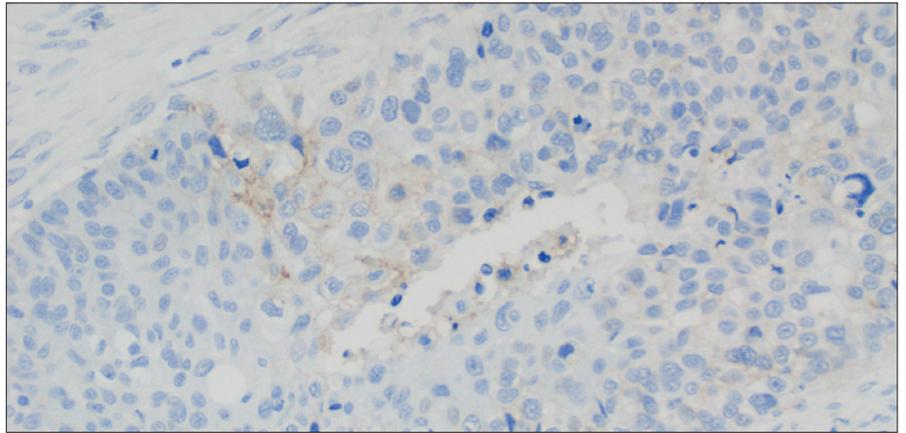


Figure 19a: NSCLC specimen stained with PD-L1 primary antibody exhibiting weak but perceptible and convincing membrane staining of tumor cells (20× magnification).

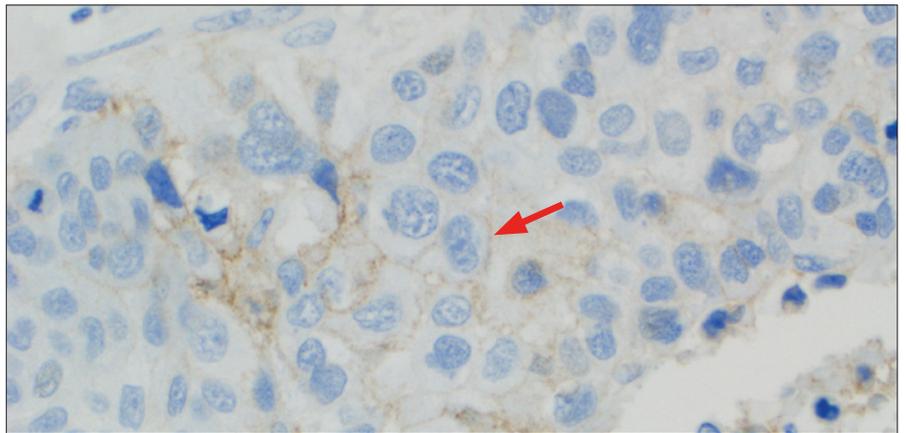


Figure 19b: NSCLC specimen stained with PD-L1 primary antibody exhibiting weak but perceptible and convincing membrane staining of tumor cells (arrow) (40× magnification).

Key Point

Weak but perceptible and convincing 1+ membrane staining of tumor cells should be included in the TPS

Distinguishing Tumor Cells From Tumor-associated Immune Cells (TAIC)

Scoring should only include all viable tumor cells with membrane staining ($\geq 1+$). Tumor-associated immune cells should be excluded from scoring.

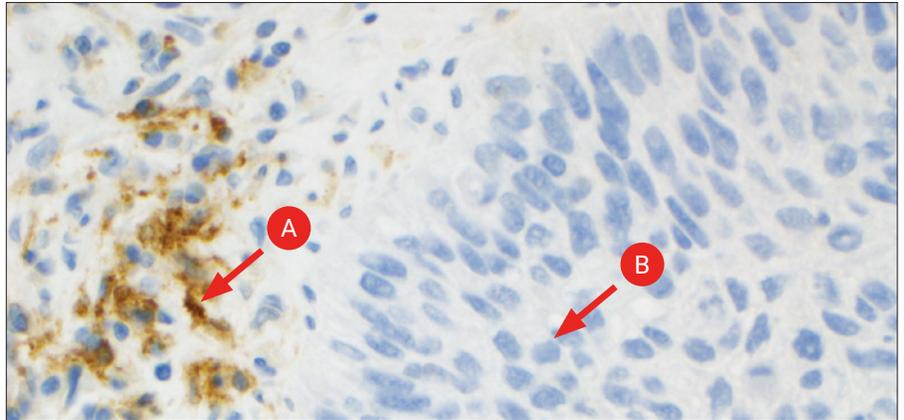


Figure 20: NSCLC specimen stained with PD-L1 primary antibody exhibiting strong staining of the TAIC (A) and lack of PD-L1 staining of tumor cells (B); TAIC staining should be excluded from the scoring (20 \times magnification).

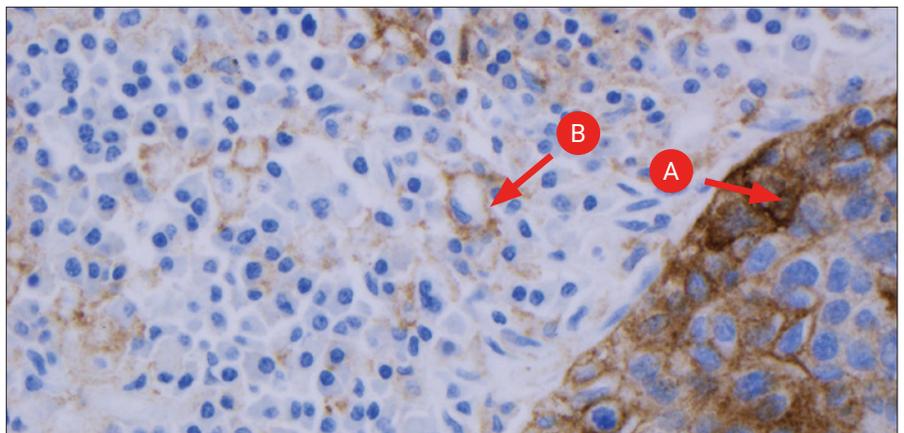


Figure 21: NSCLC specimen stained with PD-L1 primary antibody exhibiting strong staining of tumor cells (A) and moderate staining of the TAIC (B); TAIC staining should be excluded from the scoring (20 \times magnification).

Key Point

Staining of TAIC should be excluded from the TPS

Heterogeneous Staining Intensities

Membrane staining of PD-L1 on NSCLC specimens is often heterogeneous with various staining intensities (1–3+).

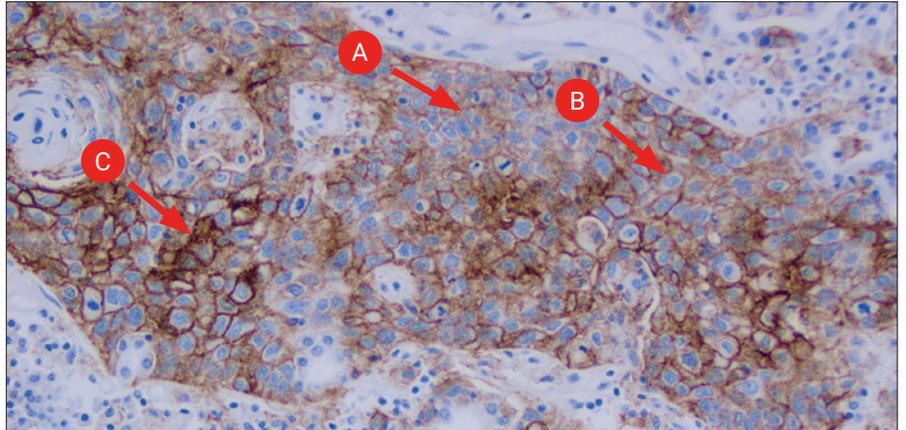


Figure 22: NSCLC specimen stained with PD-L1 primary antibody exhibiting a heterogeneous membrane staining pattern with various staining intensities: 1+ staining (A), 2+ staining (B), and 3+ staining (C) (20× magnification).

Key Point

All membrane staining of tumor cells, at all intensities (1–3+), should be included in the TPS

Partial vs. Complete Membrane Staining

Scoring should include viable tumor cells showing partial or complete membrane staining ($\geq 1+$).

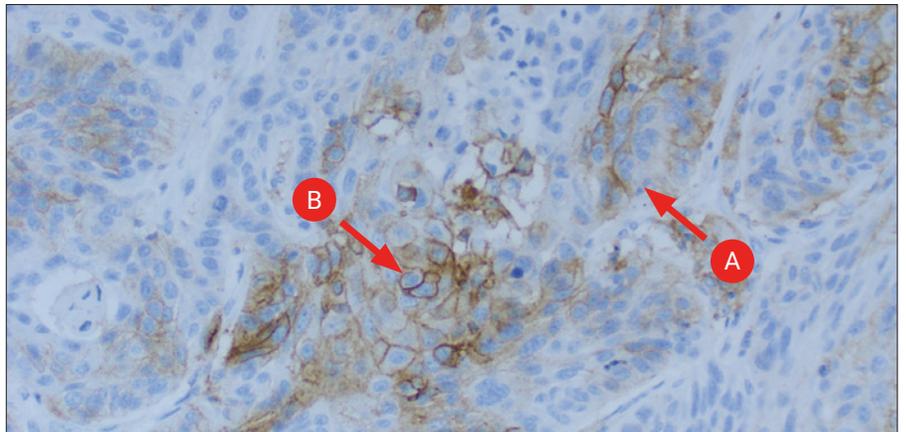


Figure 23: NSCLC specimen stained with PD-L1 primary antibody exhibiting a heterogeneous membrane staining pattern with various staining intensities (1–3+): partial membrane staining of tumor cell (A) and complete cell membrane staining (B) (20× magnification).

Key Point

Partial and/or complete membrane staining of tumor cells ($\geq 1+$) should be included in the TPS

Cytoplasmic and Membrane Staining

Tumor cells can exhibit cytoplasmic and/or membrane staining. Cytoplasmic staining should be excluded from the TPS scoring assessment.

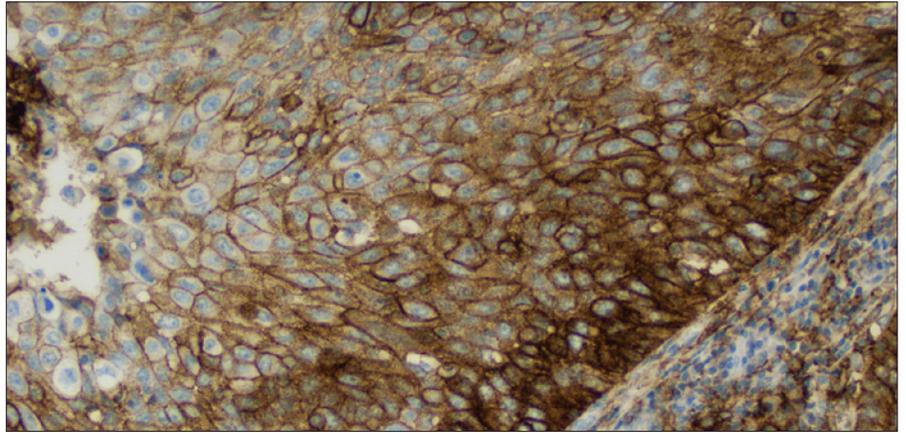


Figure 24: NSCLC specimen stained with PD-L1 primary antibody exhibiting strong cytoplasmic and membrane staining of tumor cells (20× magnification).

Key Point

Only membrane staining of tumor cells should be included in the TPS

Granular Staining

PD-L1 membrane staining may be indistinguishable when the staining pattern appears granular. Granular staining can be difficult to interpret and easily confused with cytoplasmic staining. Only perceptible and convincing granular membrane staining should be included in the TPS scoring.

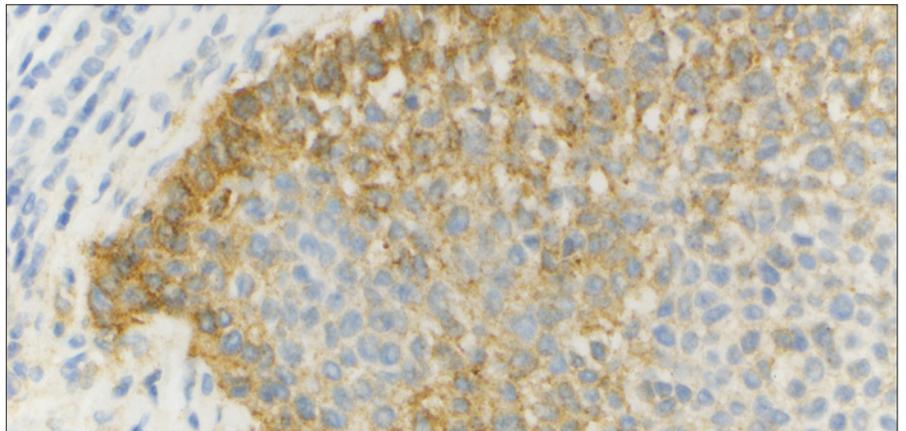


Figure 25: NSCLC specimen stained with PD-L1 primary antibody with the majority of tumor cells exhibiting a granular pattern of perceptible and convincing membrane staining (20× magnification).

Key Point

Granular staining of tumor cells must demonstrate a perceptible and convincing membrane pattern to be included in the TPS

Patchy Staining

Staining of PD-L1 on NSCLC specimens may be patchy in appearance. A review of each portion of the specimen at high power may be needed to score accurately.

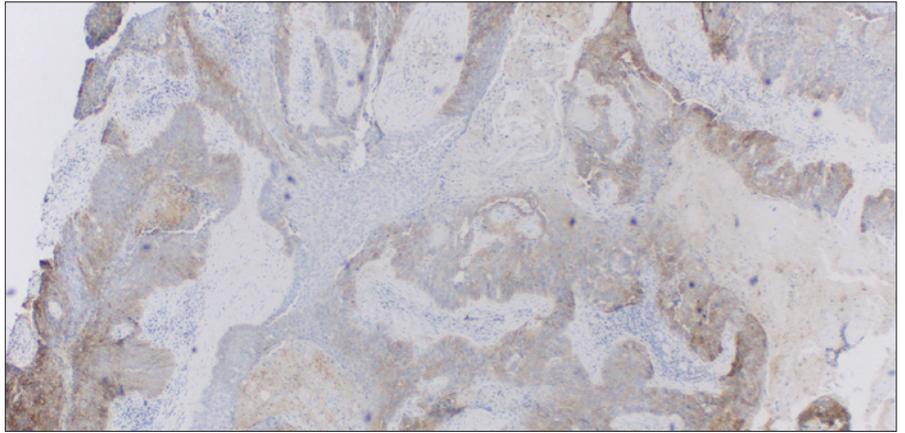


Figure 26: NSCLC specimen stained with PD-L1 primary antibody exhibiting a patchy membrane staining pattern (10× magnification).

Key Point

Assess entire specimen to accurately determine the TPS

Anthracotic Pigment

Anthracotic pigment is an accumulation of carbon in the lungs from inhaled smoke or coal dust. It appears as granular dark spots and is often helpful to distinguish tumor cells from TAIC, as anthracotic pigment is found within pulmonary macrophages and not within tumor cells.

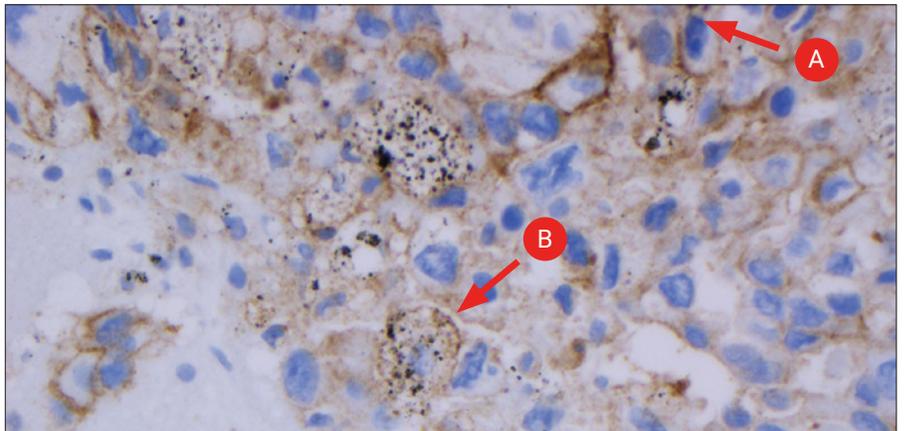


Figure 27: NSCLC specimen stained with PD-L1 primary antibody exhibiting strong staining of tumor cells (A) and moderate staining of the TAIC (B); TAIC staining should be excluded from the scoring (20× magnification).

Key Point

Anthracotic pigment should be disregarded

PD-L1 IHC 22C3 pharmDx TPS < 1% Case Examples

Case 1: TPS < 1%

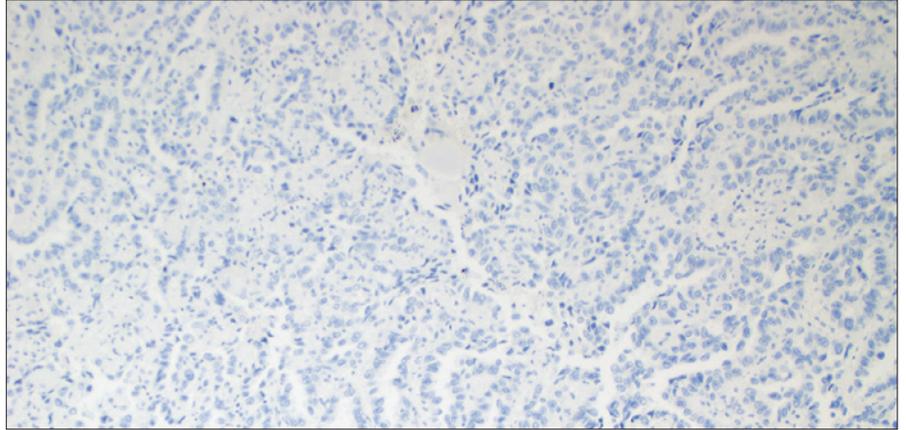


Figure 28a: 10× magnification.

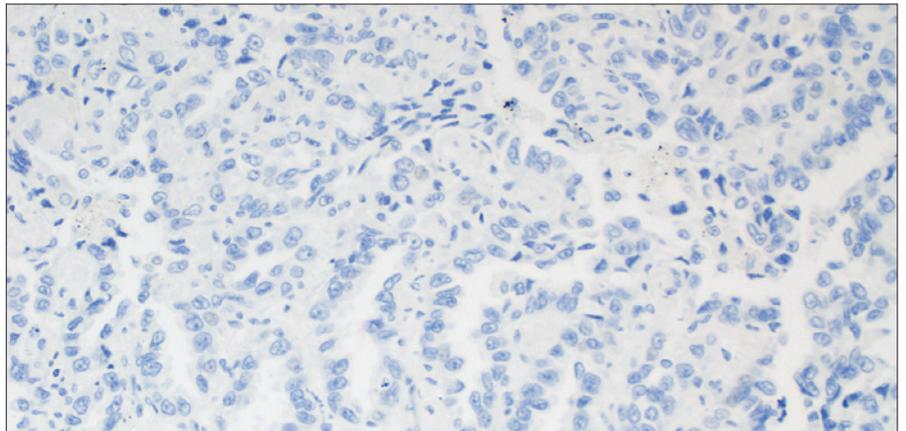


Figure 28b: 20× magnification.

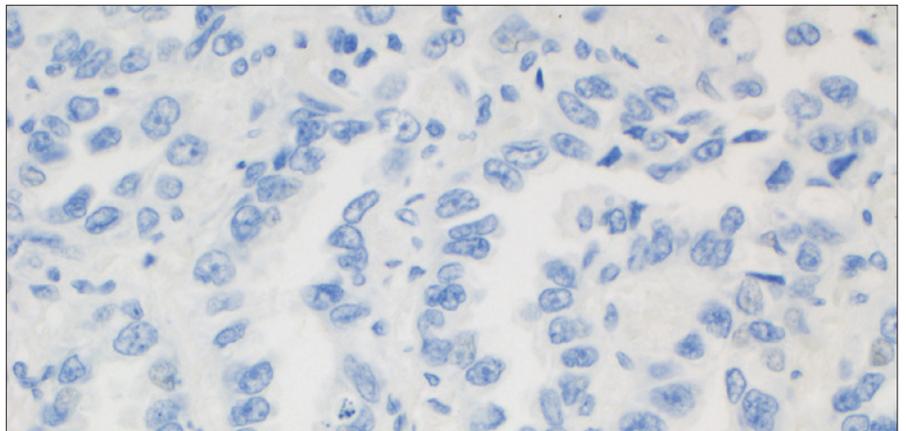


Figure 28c: 40× magnification.

Figure 28a–28c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS < 1%.

Case 2: TPS < 1%

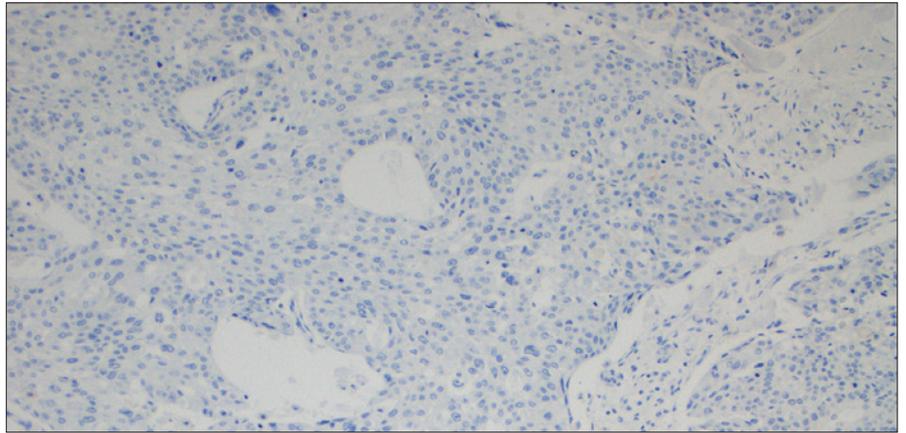


Figure 29a: 10× magnification.

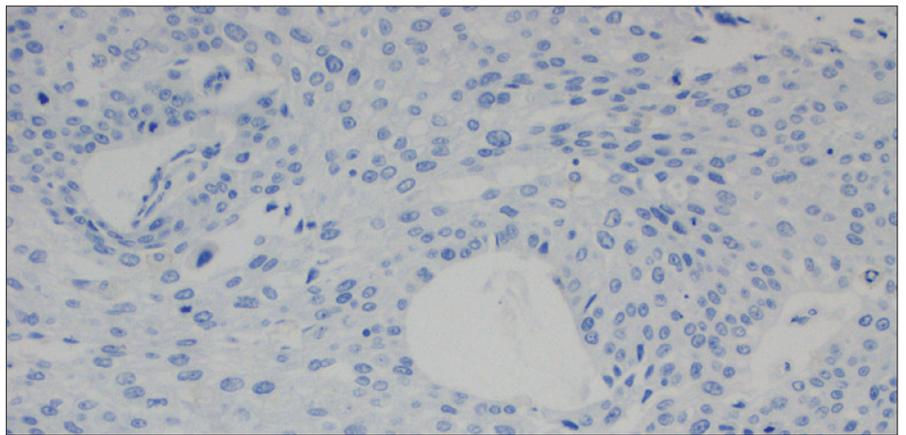


Figure 29b: 20× magnification.

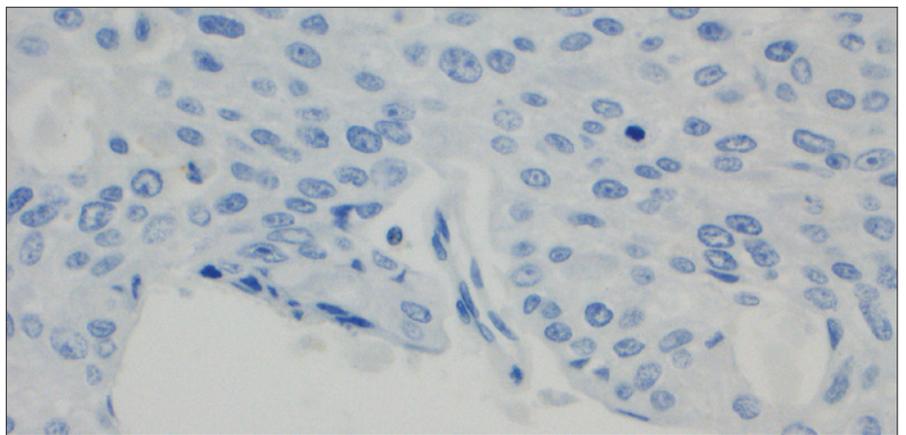


Figure 29c: 40× magnification.

Figure 29a–29c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS < 1%.

Case 3: TPS < 1%

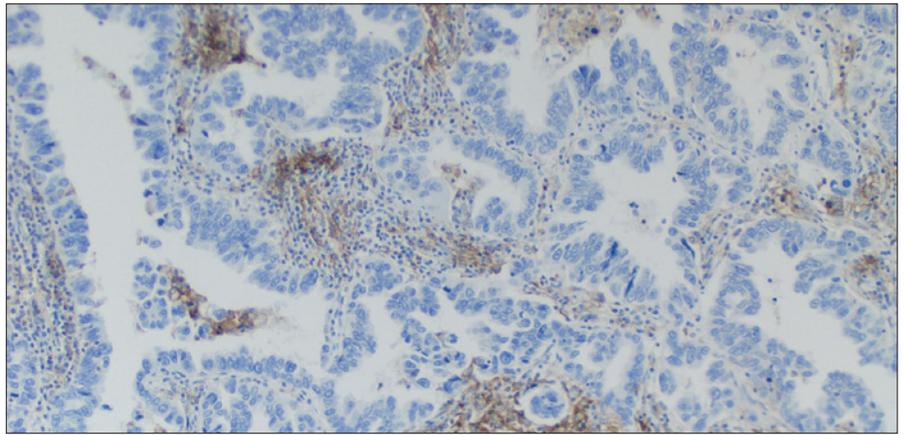


Figure 30a: 10× magnification.

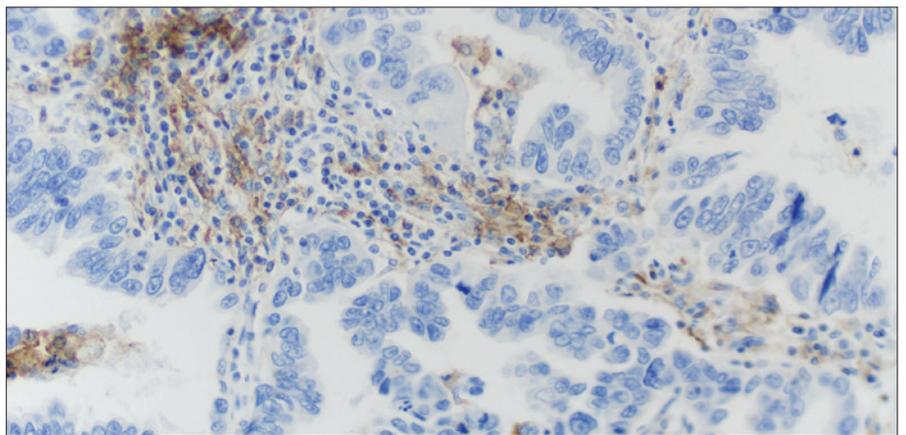


Figure 30b: 20× magnification.

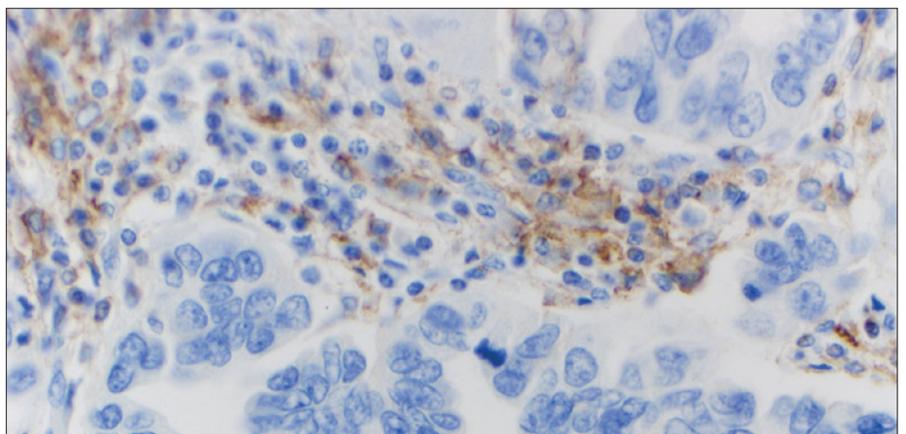


Figure 30c: 40× magnification.

Figure 30a–30c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS < 1%. TAIC are staining, but should be excluded from scoring.

Case 4: TPS < 1%

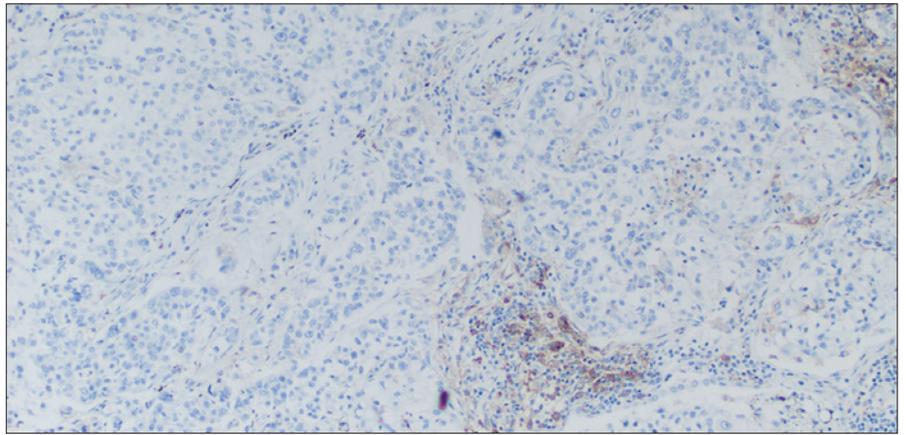


Figure 31a: 10× magnification.

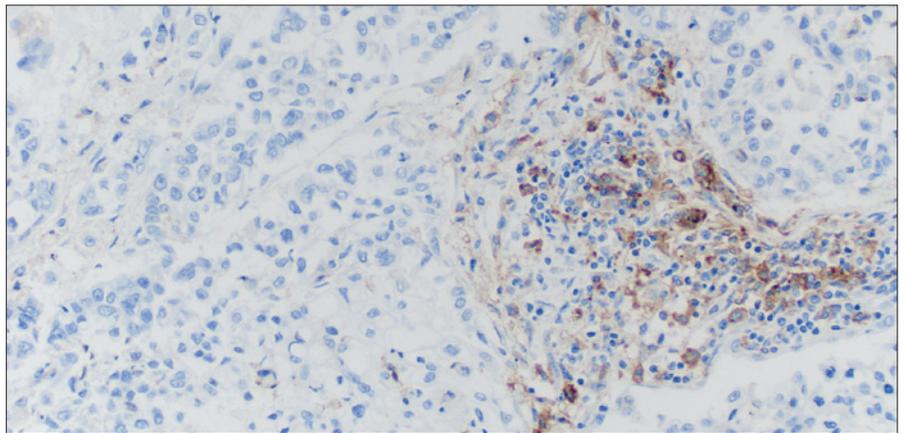


Figure 31b: 20× magnification.

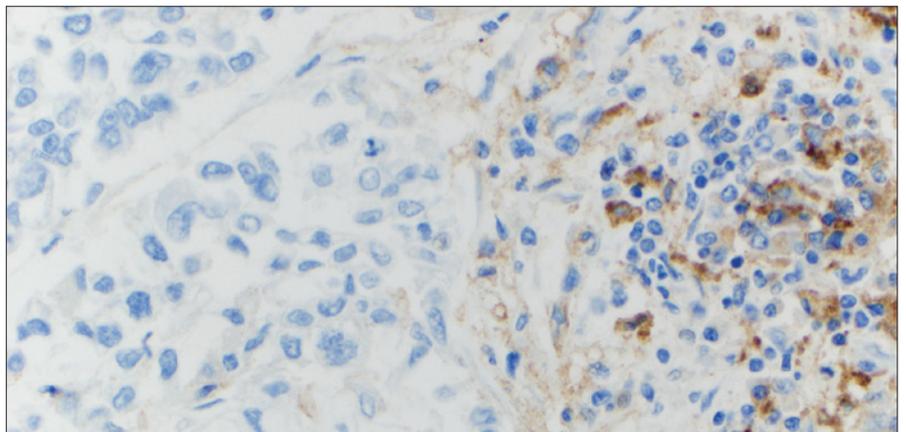


Figure 31c: 40× magnification.

Figure 31a–31c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS < 1%. TAIC are staining, but should be excluded from scoring.

PD-L1 IHC 22C3 pharmDx TPS 0–10% Case Examples

Challenging Case 1: TPS 0–10%

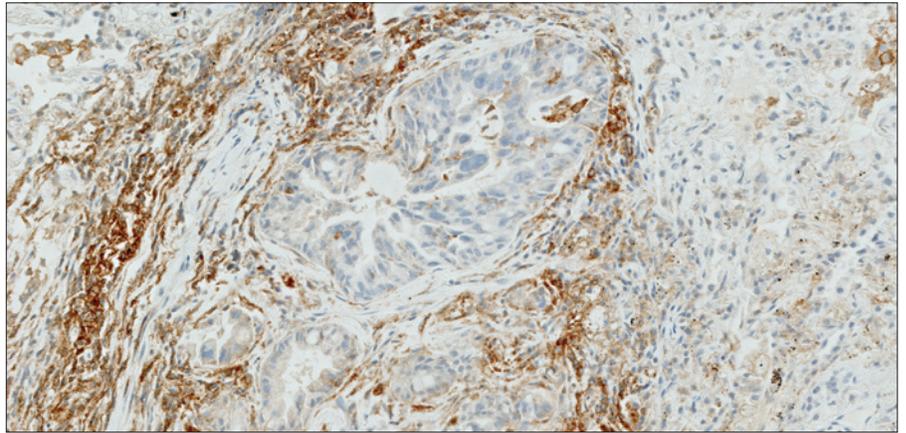


Figure 32a: 10× magnification.

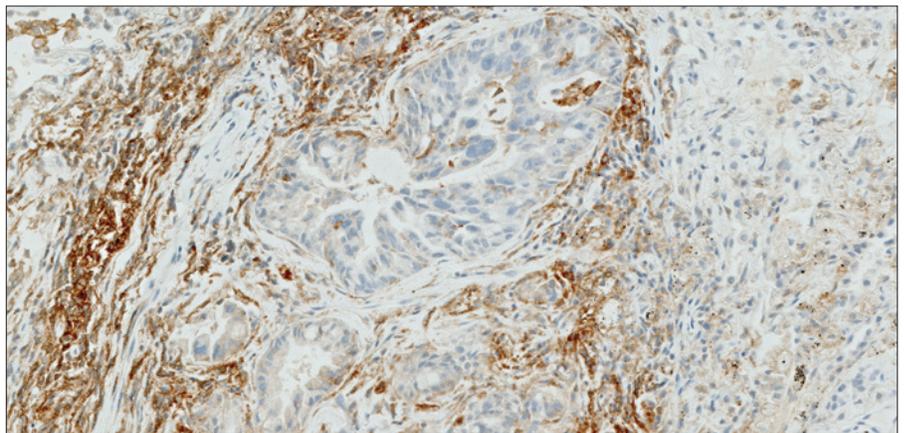


Figure 32b: 20× magnification.

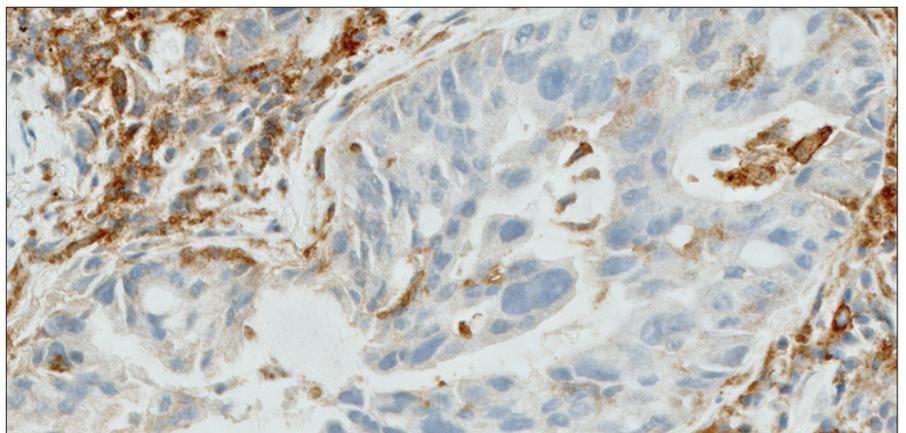


Figure 32c: 40× magnification.

Figure 32a–32c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS < 1%.

Challenging Case 2:
TPS 0–10%

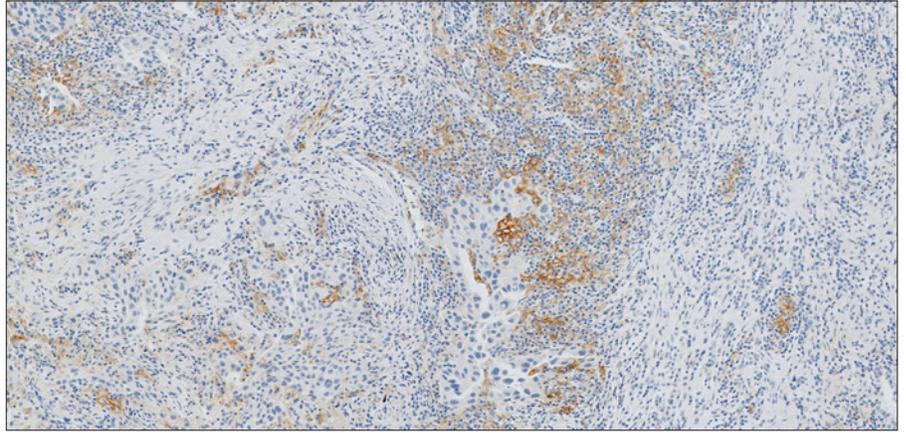


Figure 33a: 10× magnification.

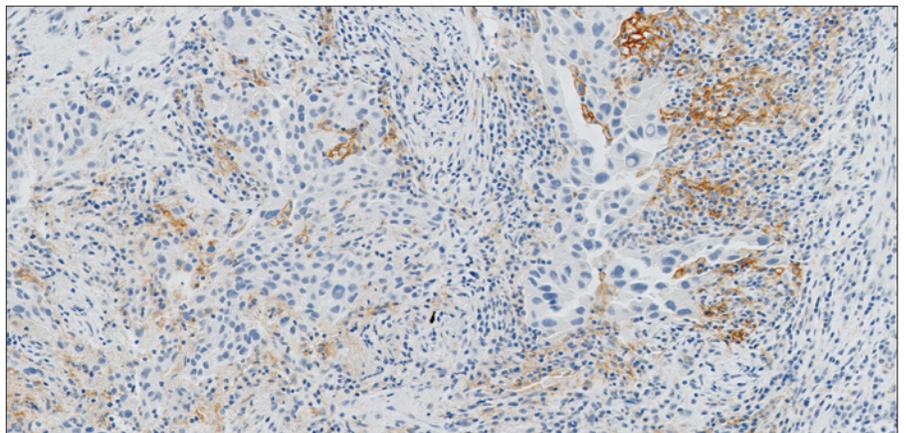


Figure 33b: 20× magnification.

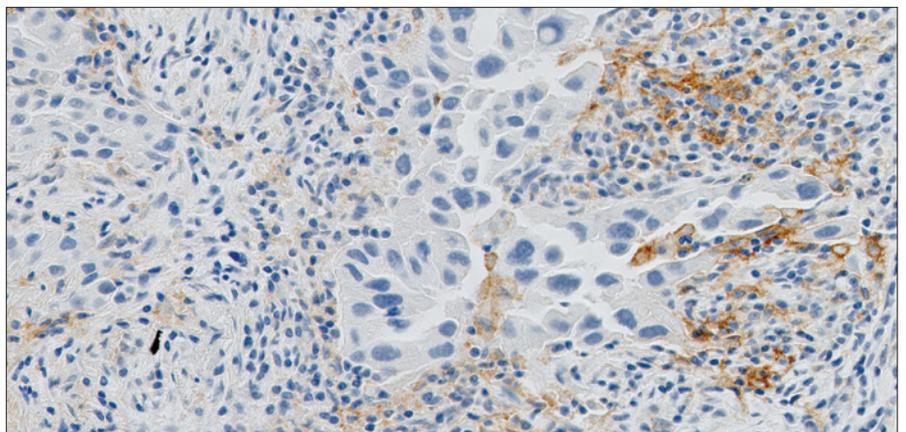


Figure 33c: 40× magnification.

Figure 33a–33c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS < 1%.

Challenging Case 3:
TPS 0–10%

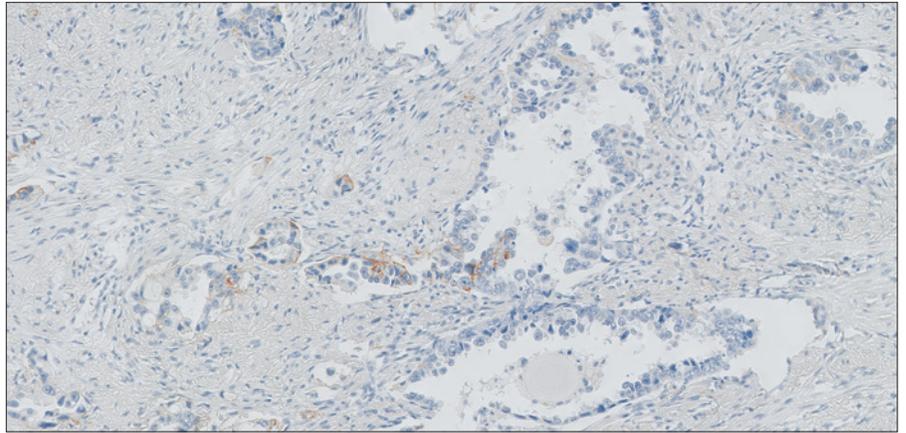


Figure 34a: 10× magnification.

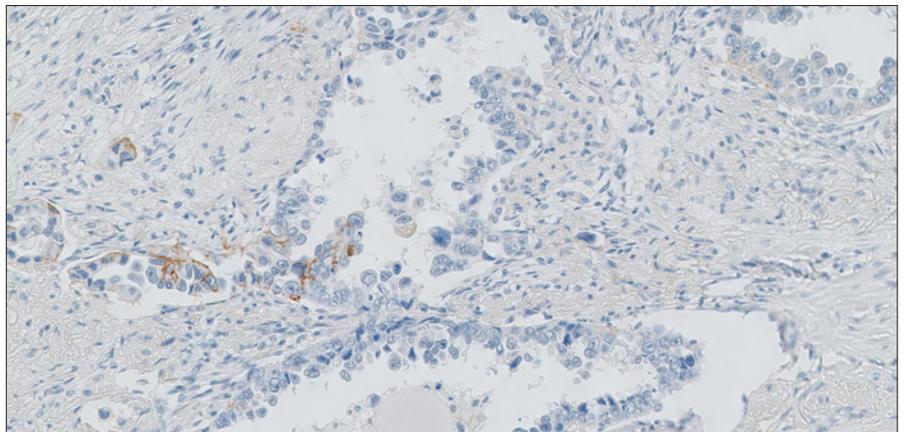


Figure 34b: 20× magnification.

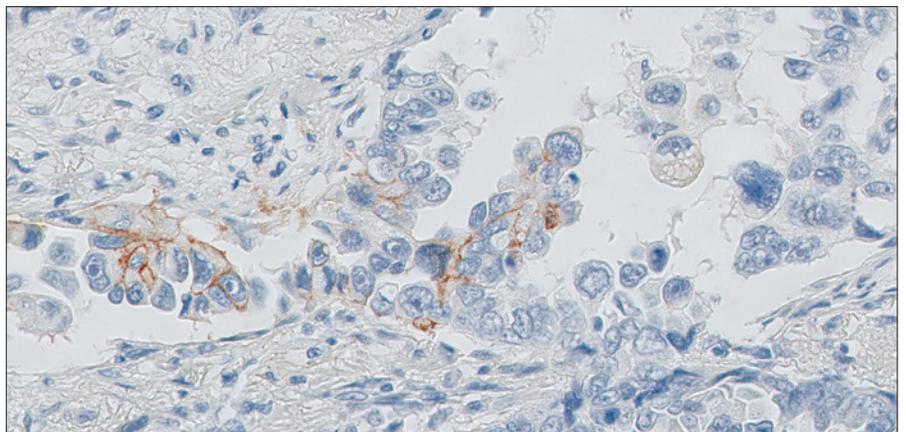


Figure 34c: 40× magnification.

Figure 34a–34c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS 1–10%.

Challenging Case 4:
TPS 0–10%

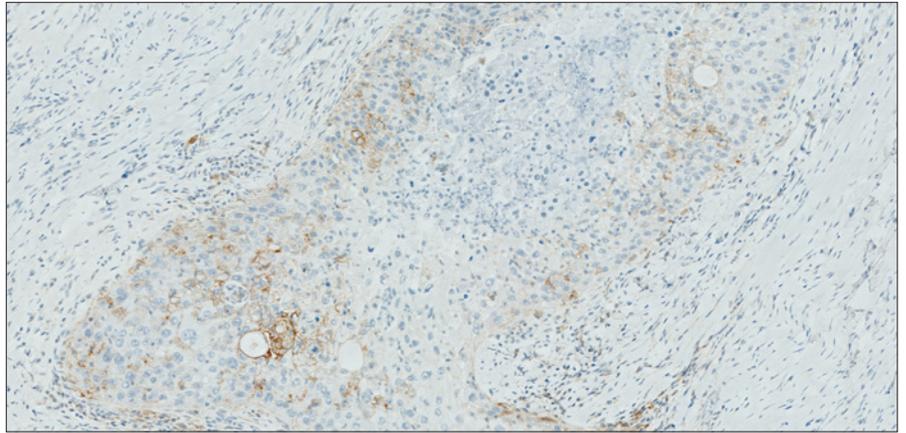


Figure 35a: 10× magnification.

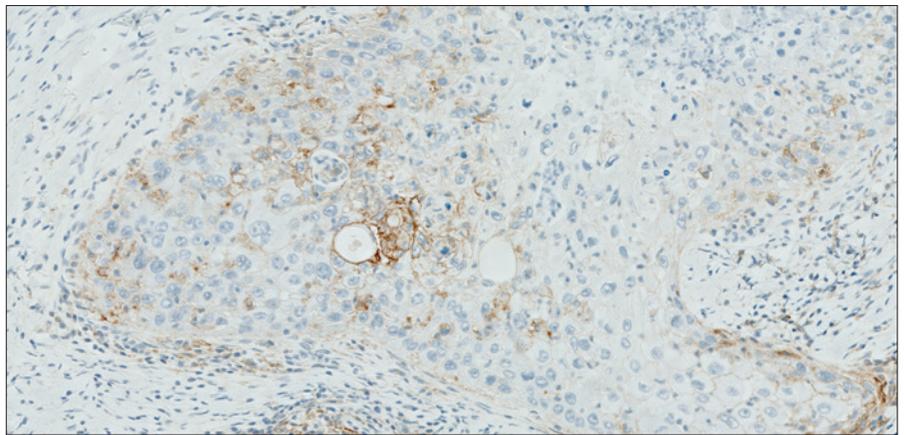


Figure 35b: 20× magnification.

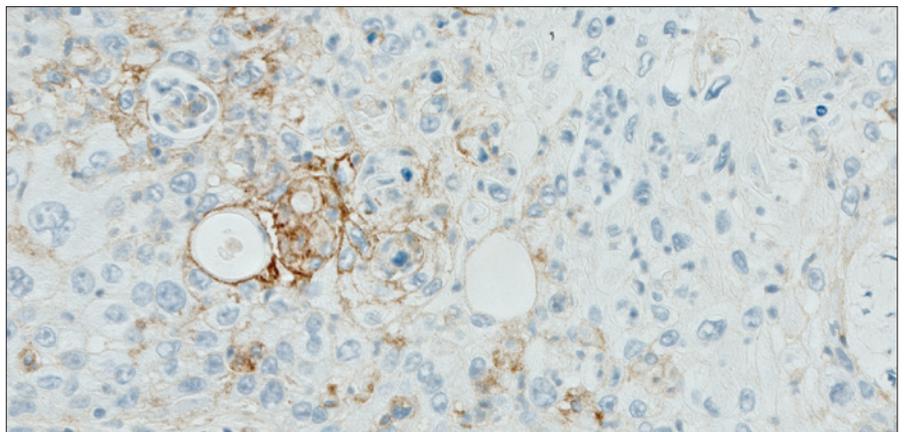


Figure 35c: 40× magnification.

Figure 35a–35c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS 1–10%.

Challenging Case 5:
TPS 0–10%

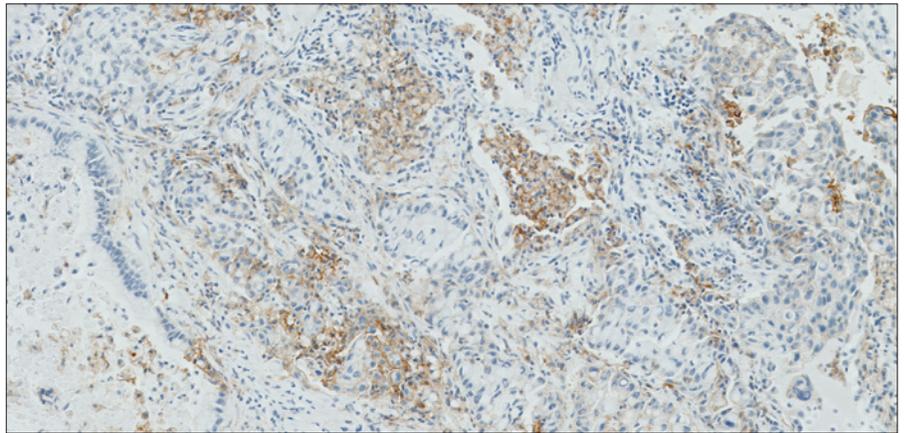


Figure 36a: 10× magnification.

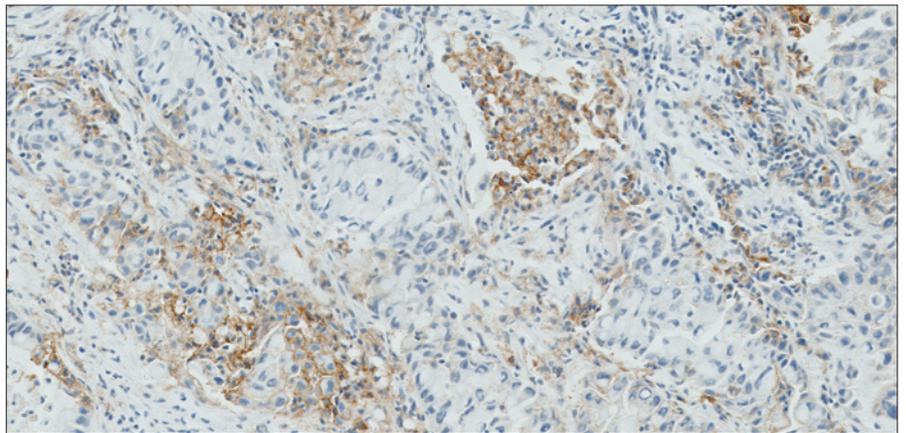


Figure 36b: 20× magnification.

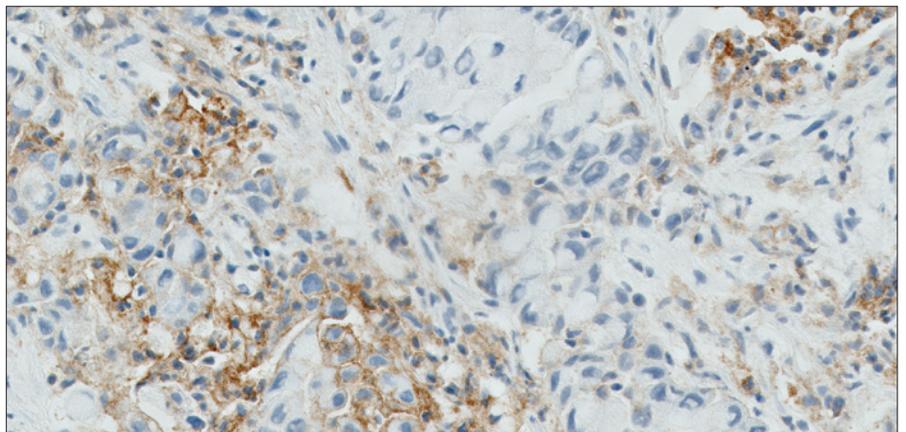


Figure 36c: 40× magnification.

Figure 36a–36c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS 1–10%.

PD-L1 IHC 22C3 pharmDx TPS 1–49% Case Examples

Case 5: TPS 1–49%

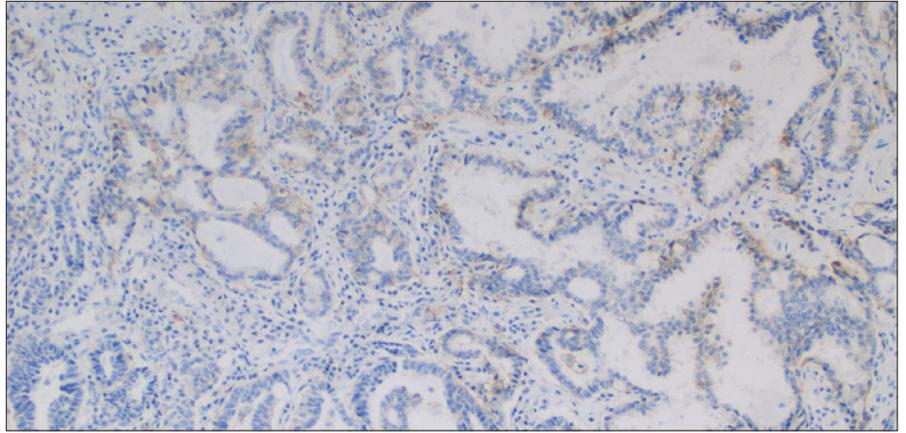


Figure 37a: 10× magnification.

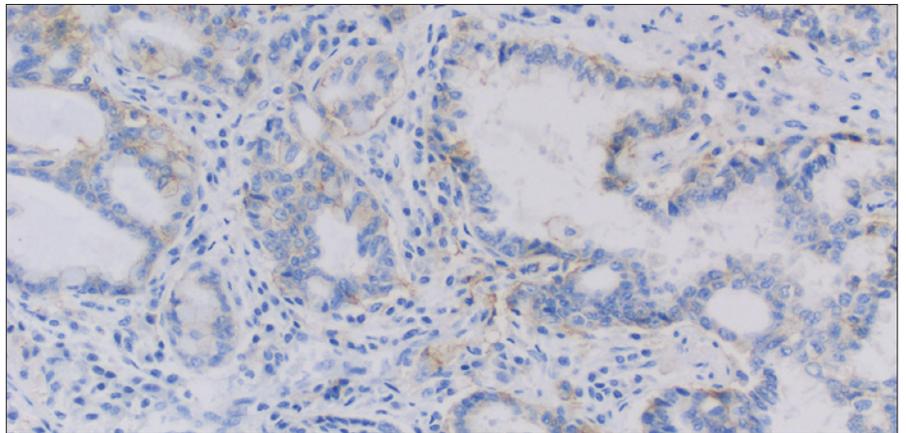


Figure 37b: 20× magnification.

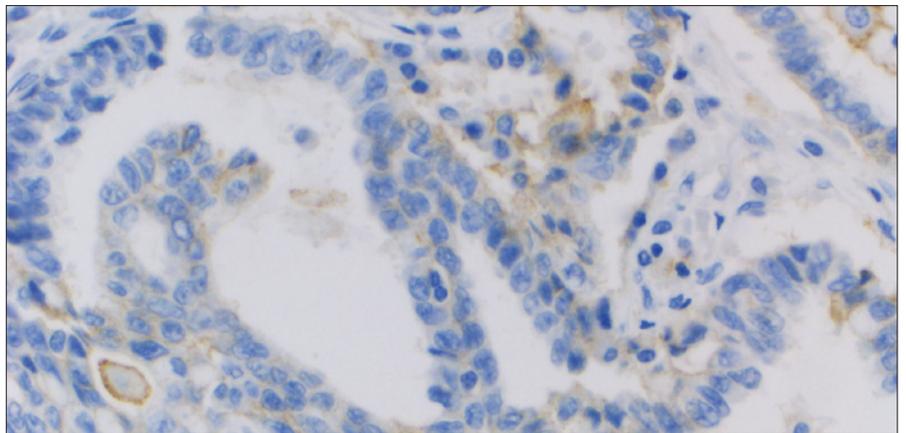


Figure 37c: 40× magnification.

Figure 37a–37c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS 1–49%.

Case 6: TPS 1–49%

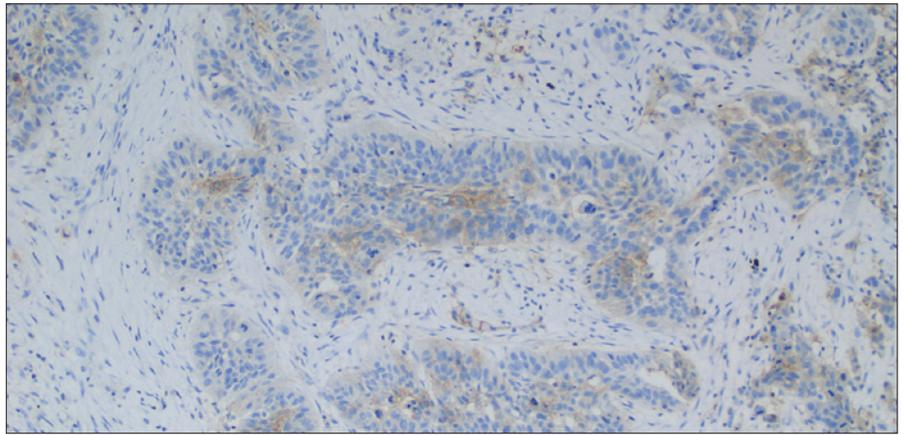


Figure 38a: 10× magnification.

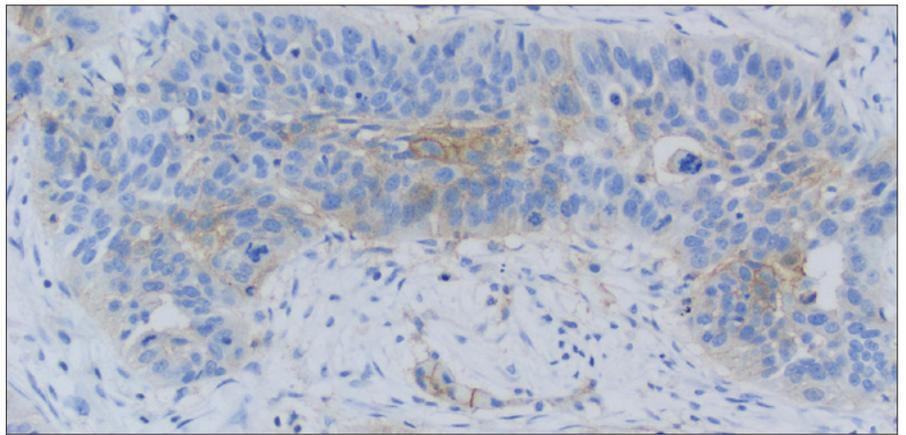


Figure 38b: 20× magnification.

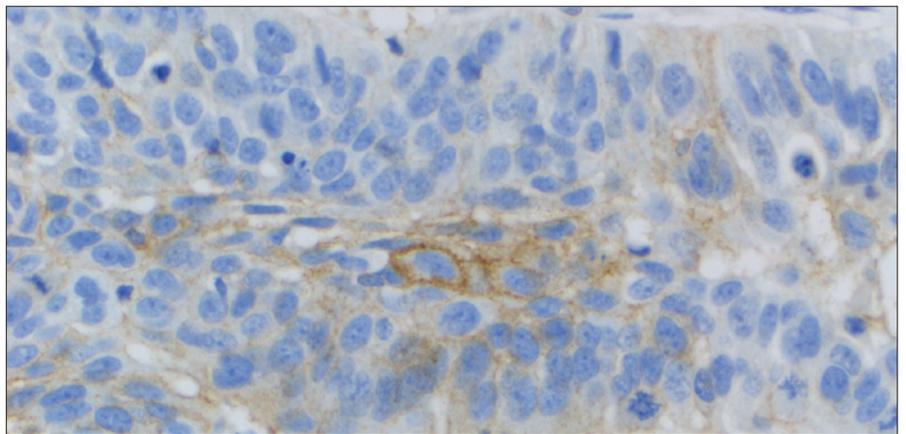


Figure 38c: 40× magnification.

Figure 38a–38c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS 1–49%.

Case 7: TPS 1–49%

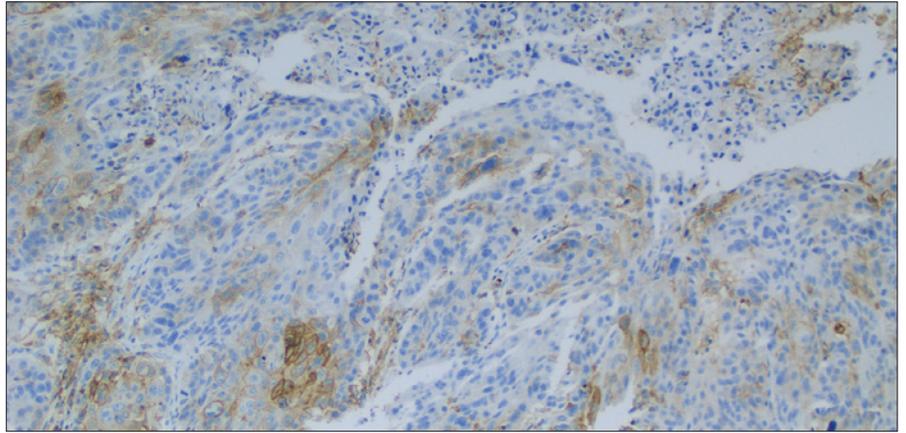


Figure 39a: 10× magnification.

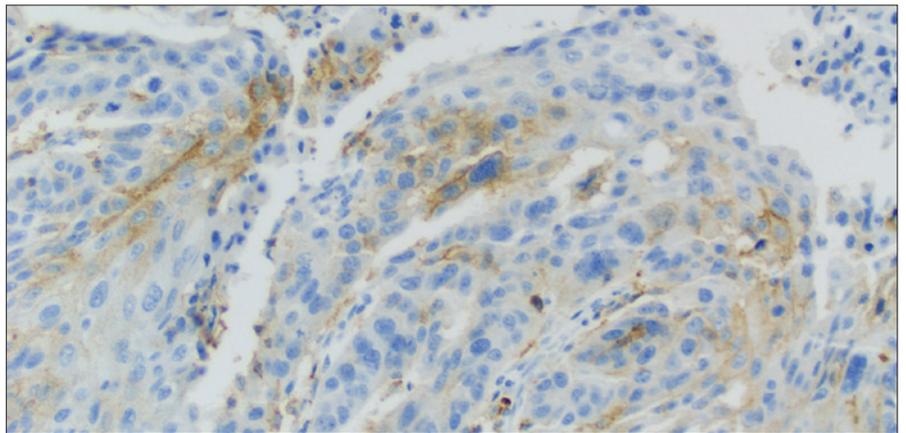


Figure 39b: 20× magnification.

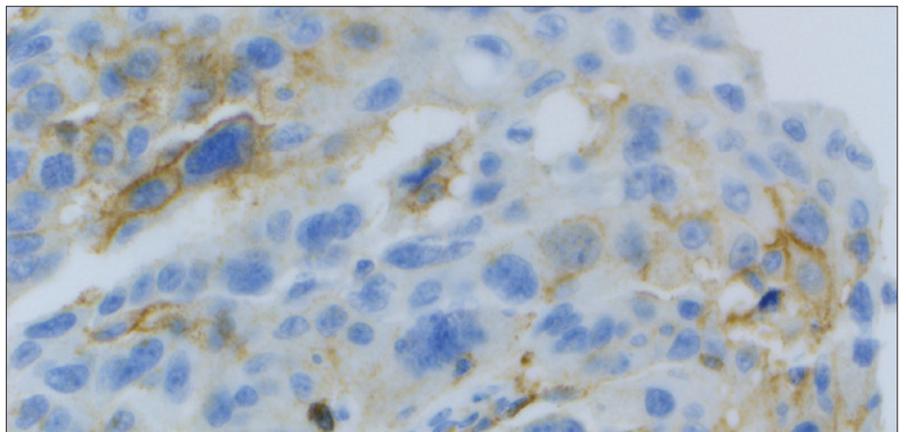


Figure 39c: 40× magnification.

Figure 39a–39c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS 1–49%.

Case 8: TPS 1–49%

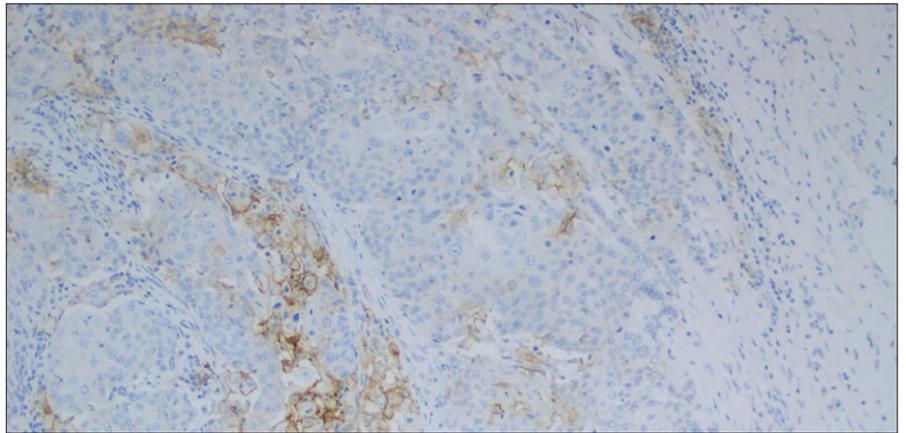


Figure 40a: 10× magnification.

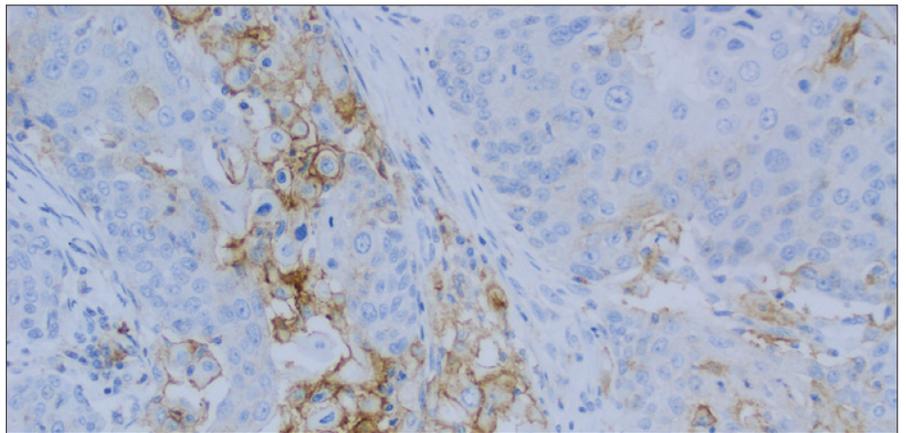


Figure 40b: 20× magnification.

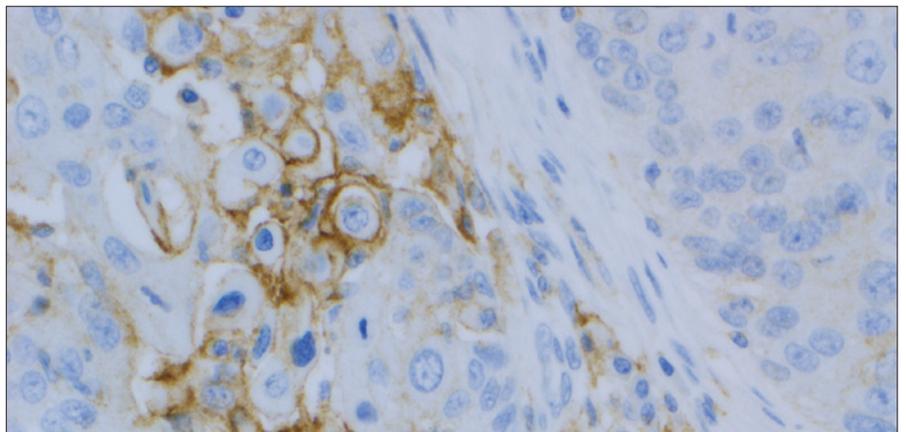


Figure 40c: 40× magnification.

Figure 40a–40c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS 1–49%.

PD-L1 IHC 22C3 pharmDx TPS \geq 50% Case Examples

Case 9: TPS \geq 50%

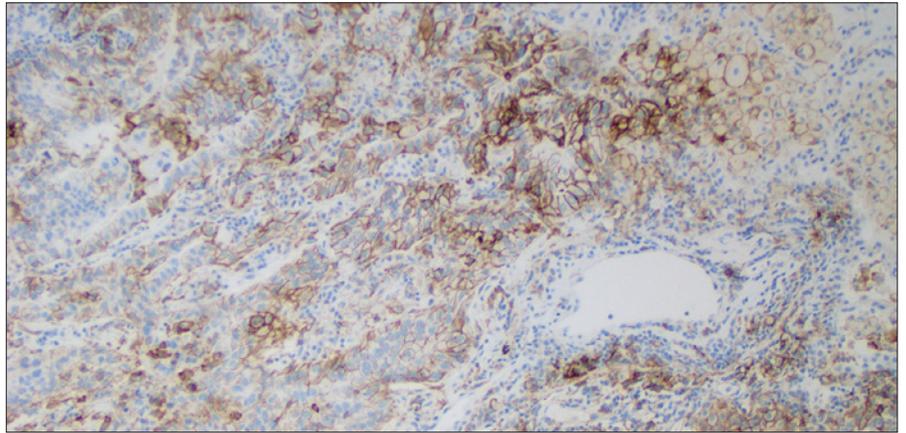


Figure 41a: 10 \times magnification.

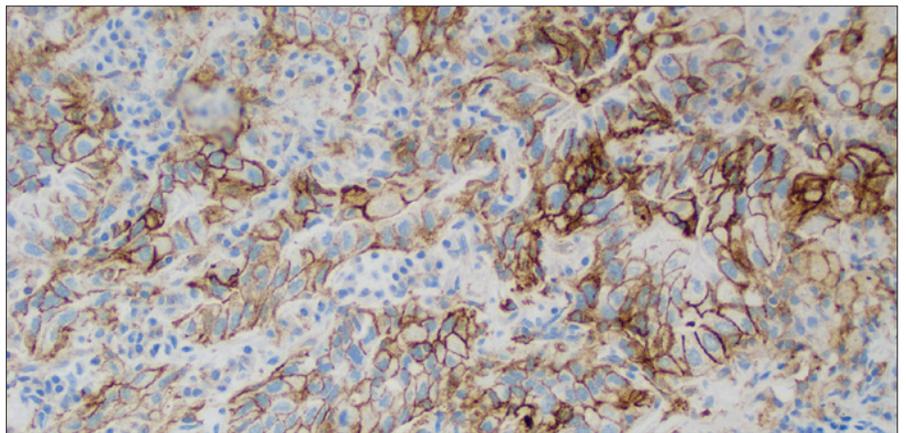


Figure 41b: 20 \times magnification.

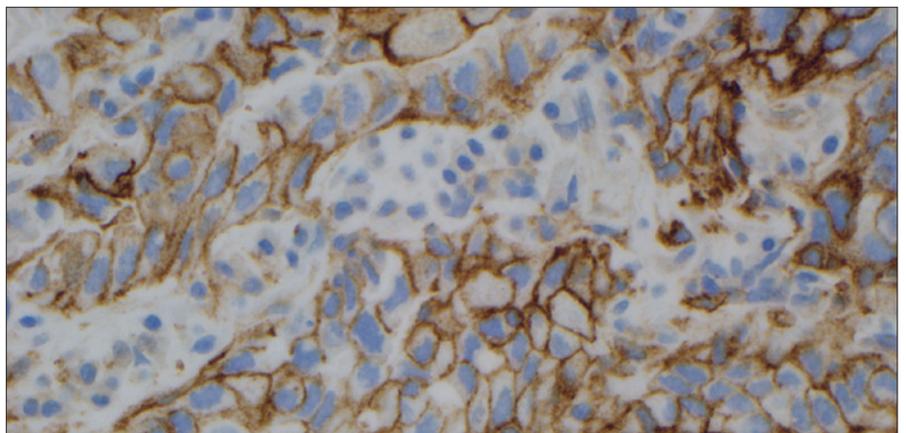


Figure 41c: 40 \times magnification.

Figure 41a–41c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS \geq 50%.

Case 10: TPS \geq 50%

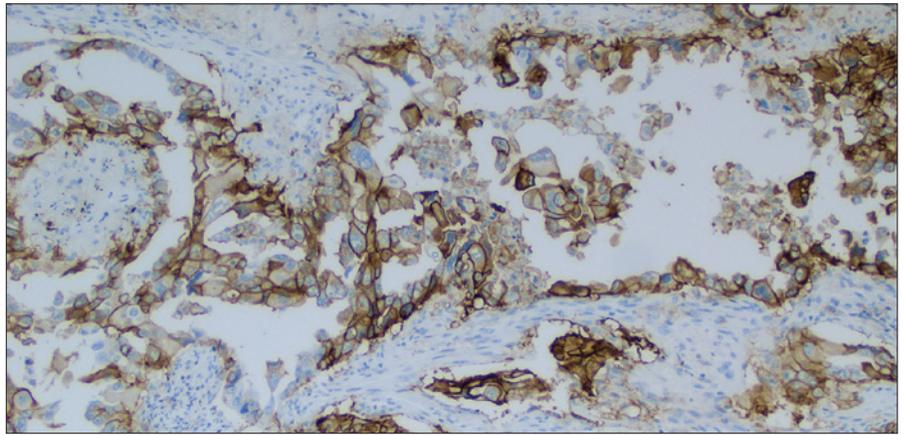


Figure 42a: 10 \times magnification.

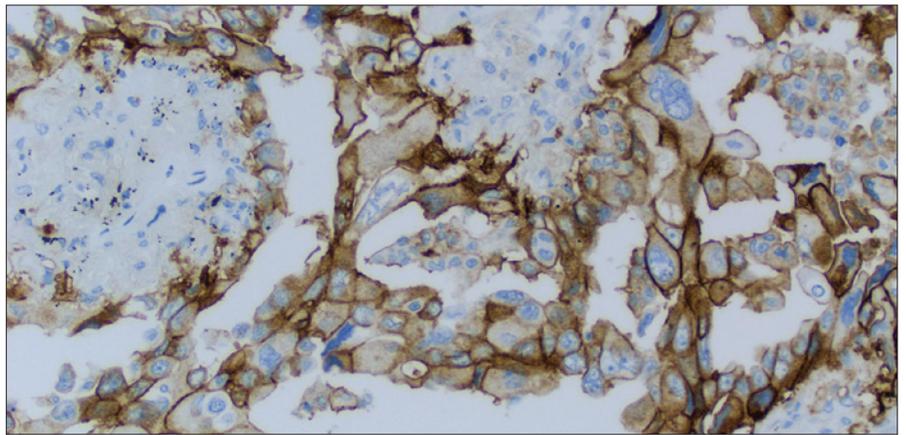


Figure 42b: 20 \times magnification.

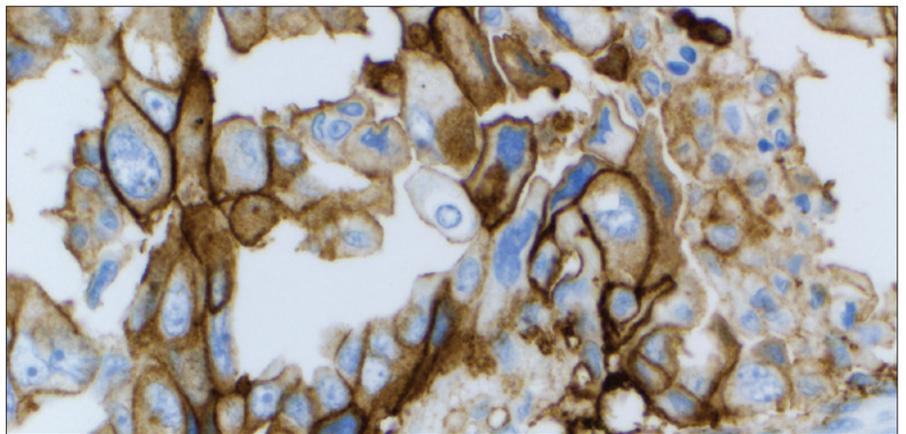


Figure 42c: 40 \times magnification.

Figure 42a–42c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS \geq 50%.

Case 11: TPS \geq 50%

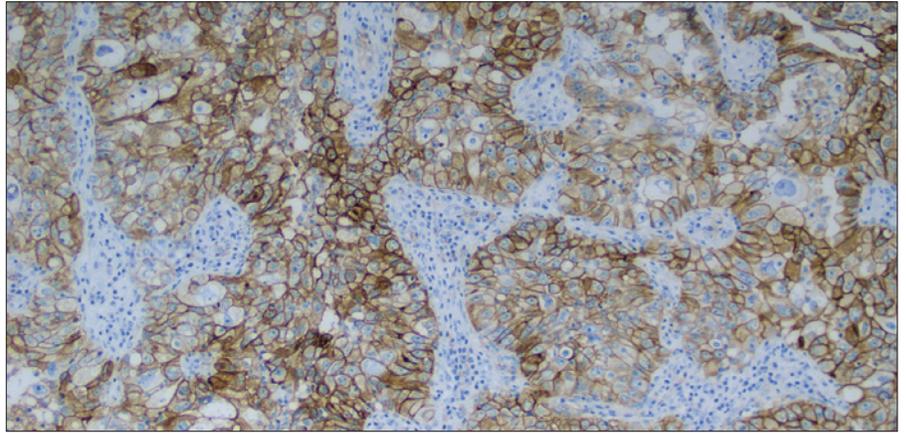


Figure 43a: 10 \times magnification.

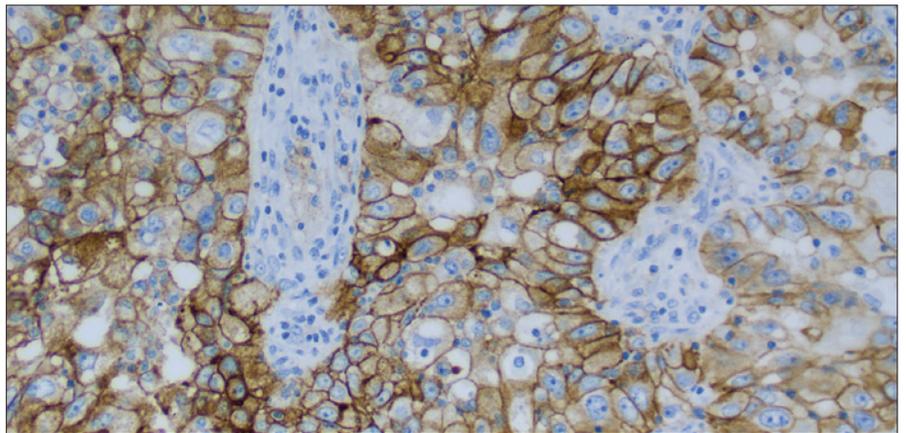


Figure 43b: 20 \times magnification.

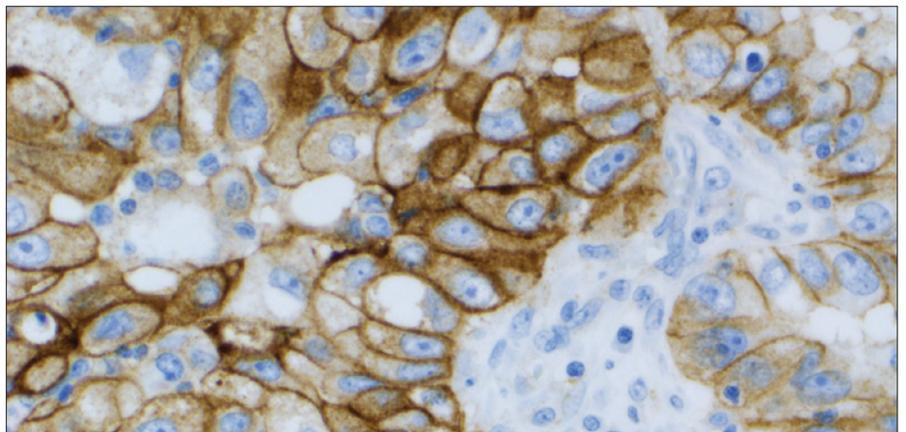


Figure 43c: 40 \times magnification.

Figure 43a–43c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS \geq 50%.

Case 12: TPS \geq 50%

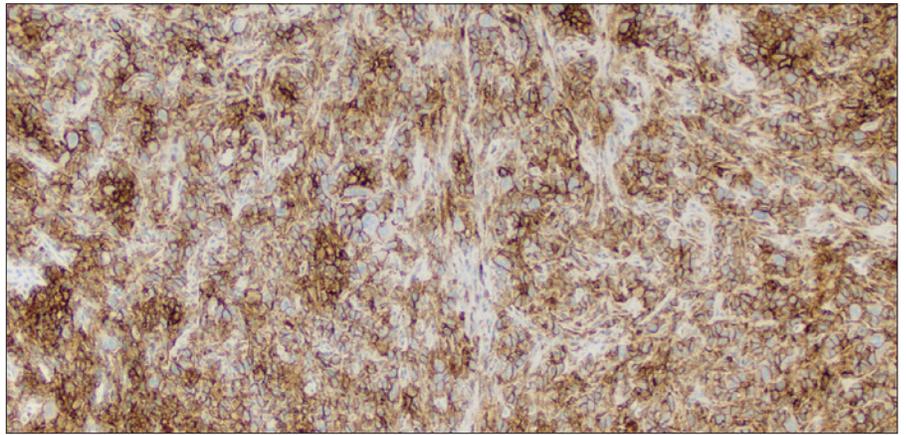


Figure 44a: 10 \times magnification.

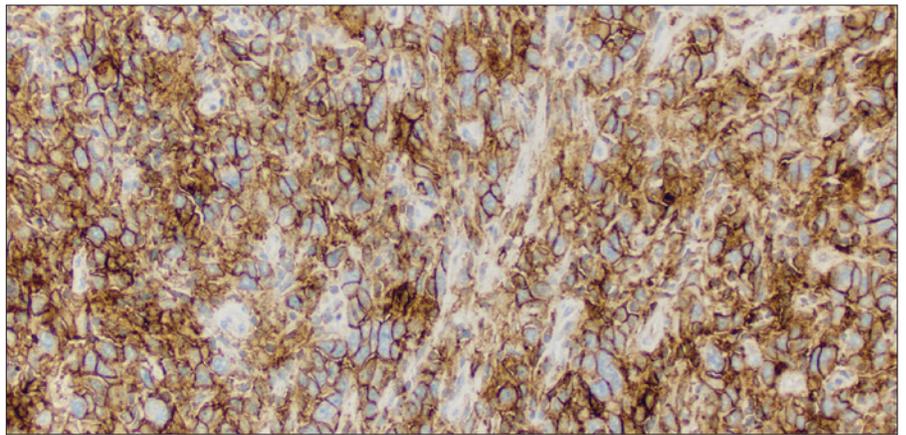


Figure 44b: 20 \times magnification.

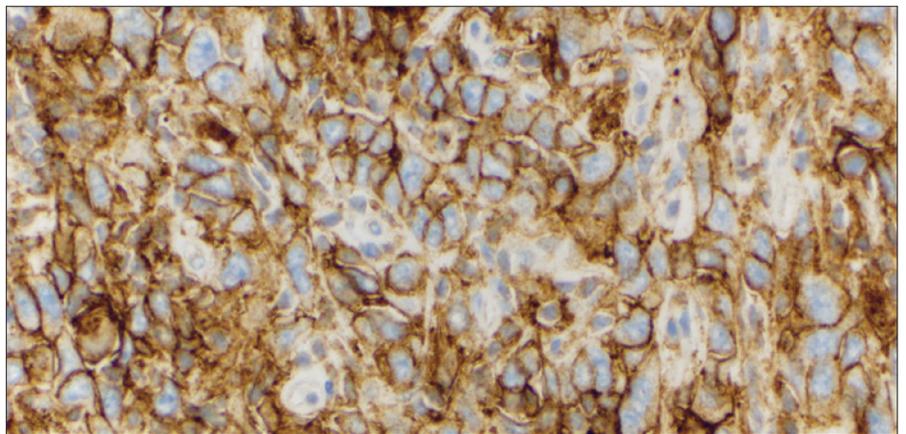


Figure 44c: 40 \times magnification.

Figure 44a–44c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS \geq 50%.

Case 13: TPS \geq 50%

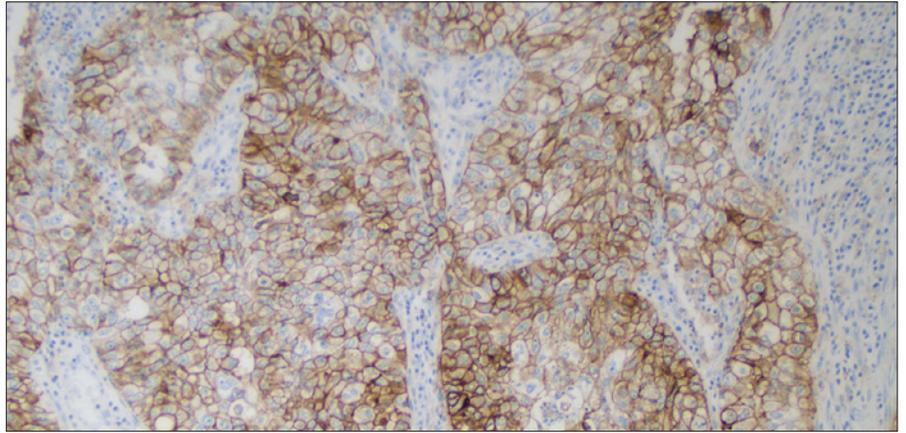


Figure 45a: 10 \times magnification.

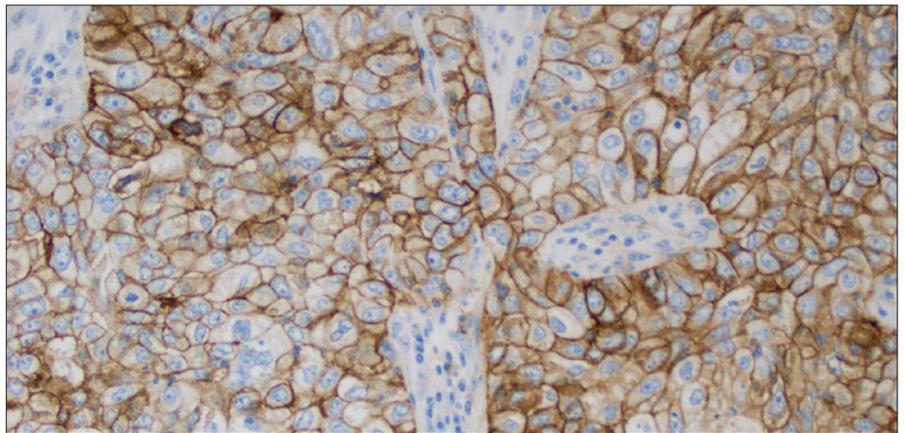


Figure 45b: 20 \times magnification.

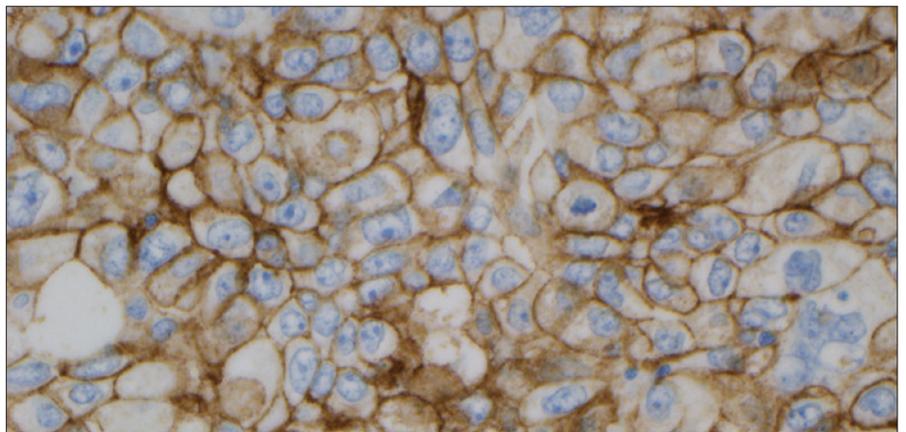


Figure 45c: 40 \times magnification.

Figure 45a–45c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS \geq 50%.

Case 14: TPS \geq 50%

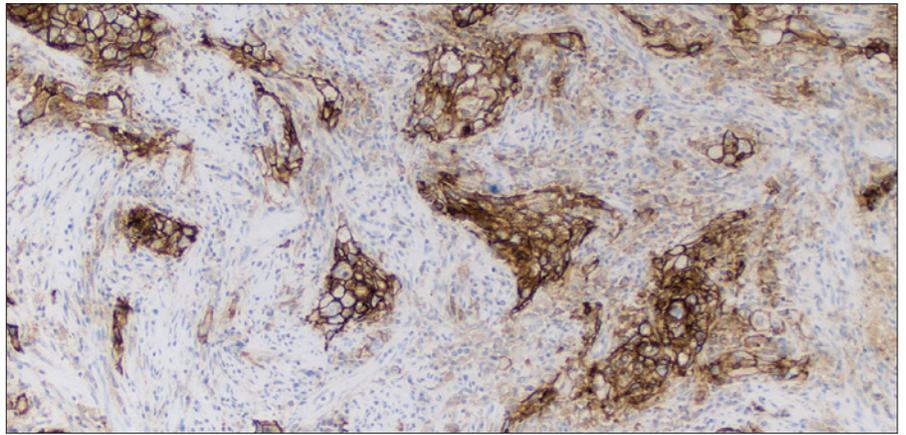


Figure 46a: 10 \times magnification.

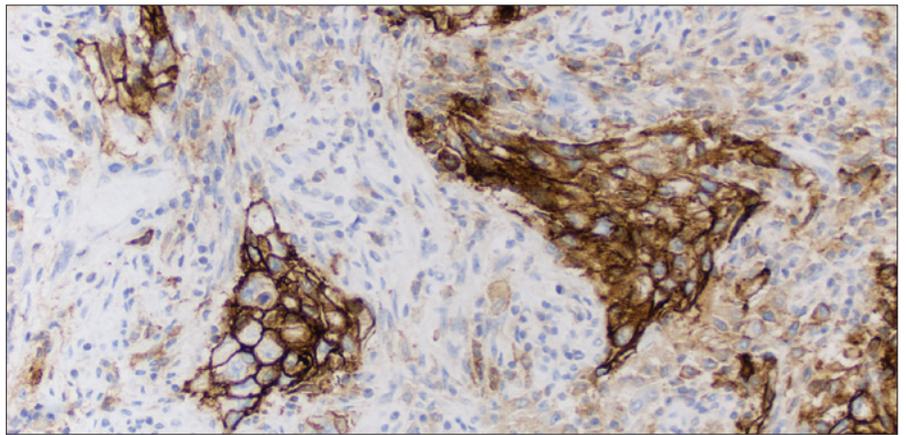


Figure 46b: 20 \times magnification.

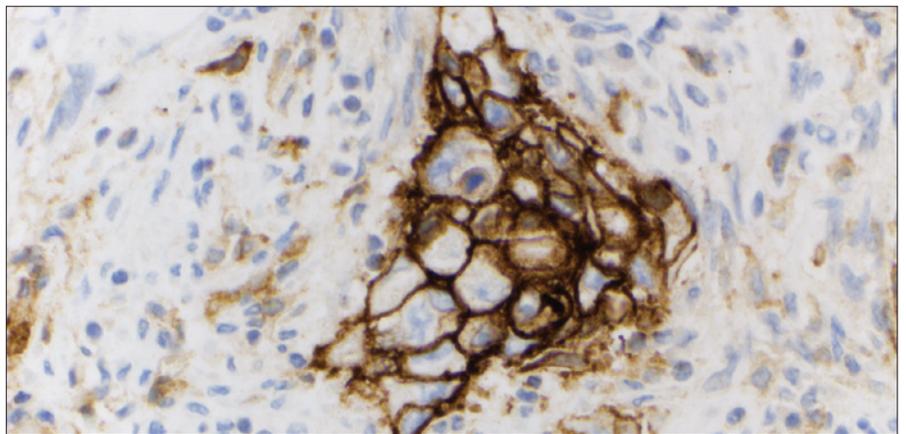


Figure 46c: 40 \times magnification.

Figure 46a–46c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS \geq 50%.

Case 15: TPS \geq 50%

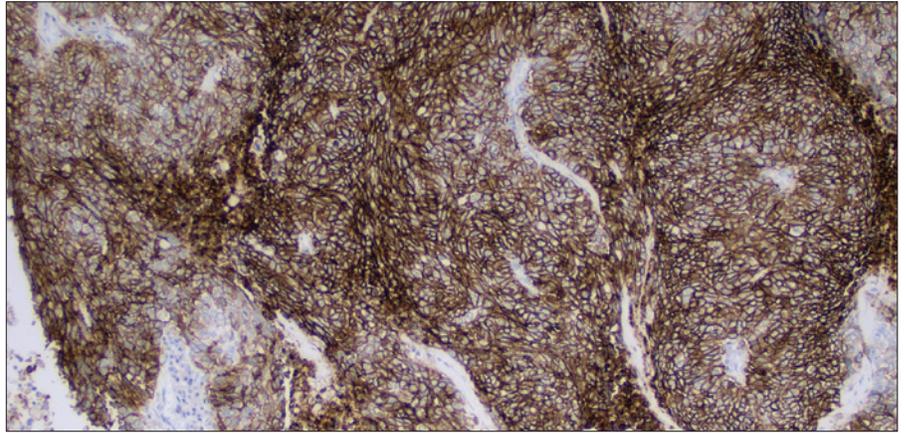


Figure 47a: 10 \times magnification.

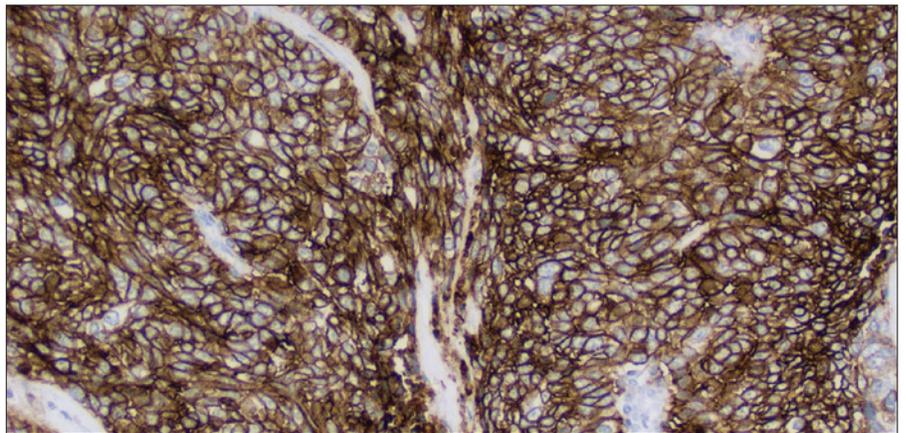


Figure 47b: 20 \times magnification.

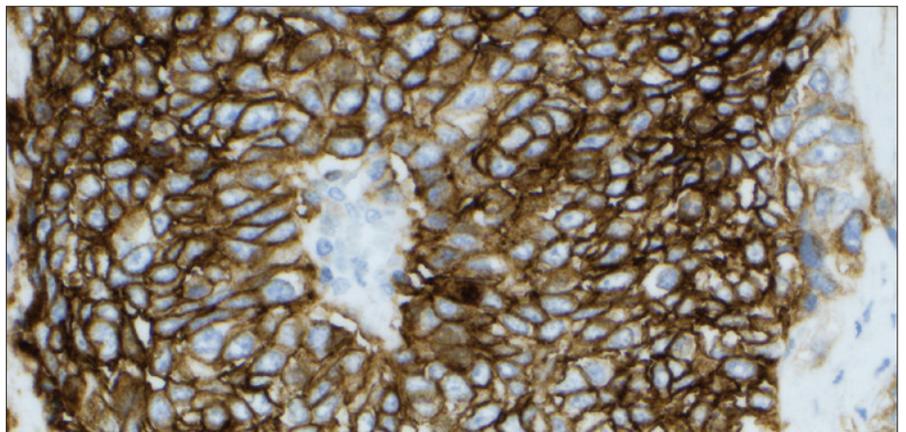


Figure 47c: 40 \times magnification.

Figure 47a–47c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS \geq 50%.

PD-L1 IHC 22C3 pharmDx TPS 40–60% Case Examples

Challenging Case 6: TPS 40–60%

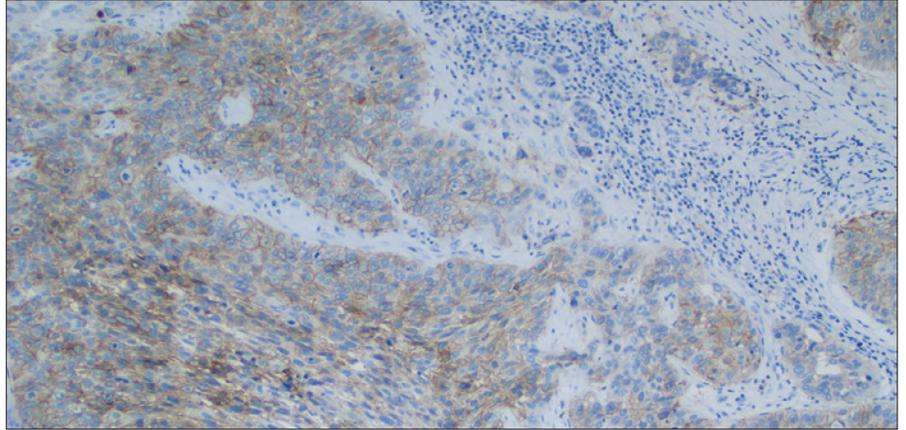


Figure 48a: 10× magnification.

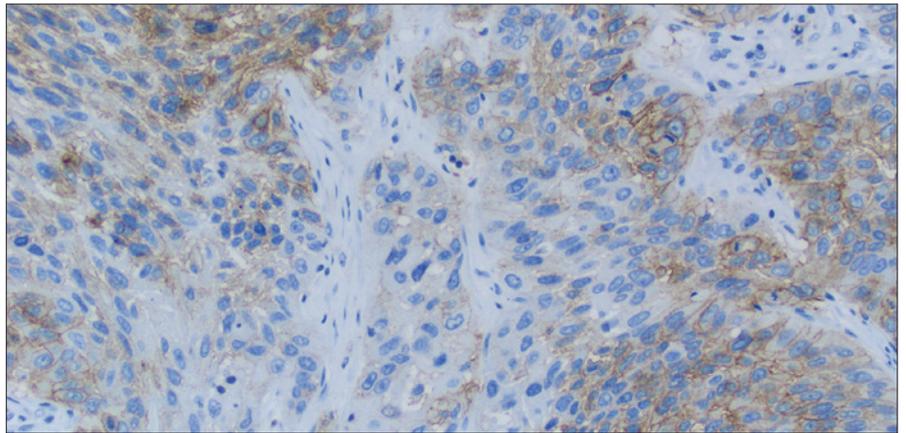


Figure 48b: 20× magnification.

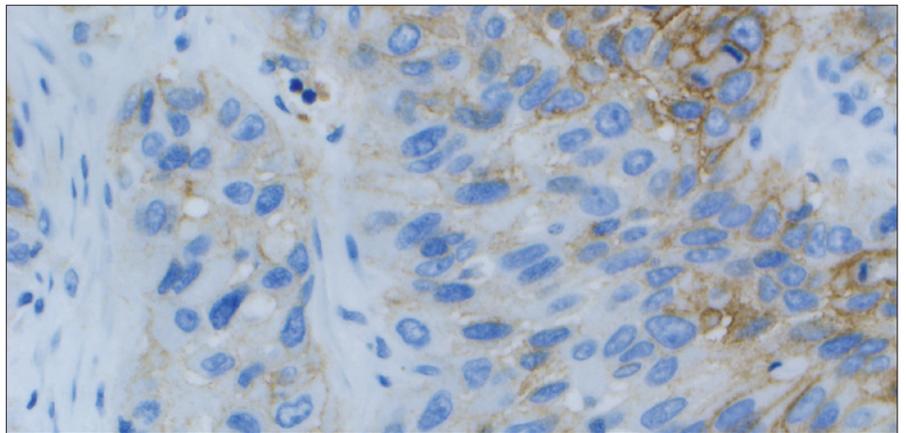


Figure 48c: 40× magnification.

Figure 48a–48c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS 40%.

Challenging Case 7:
TPS 40–60%

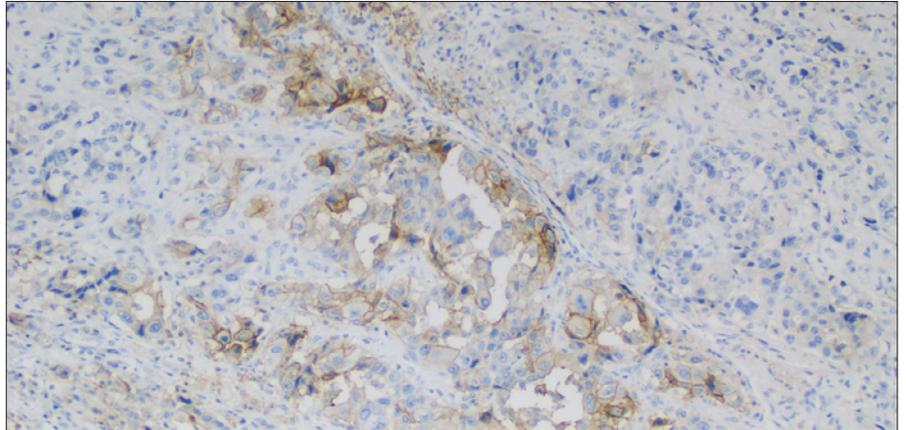


Figure 49a: 10× magnification.

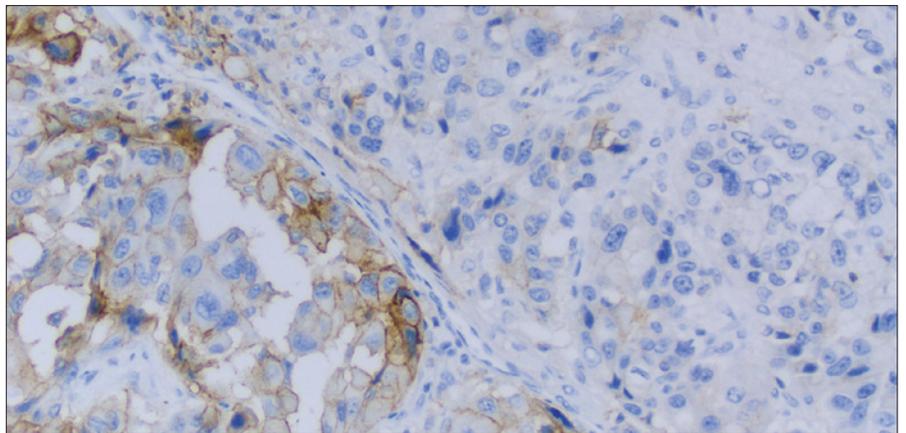


Figure 49b: 20× magnification.

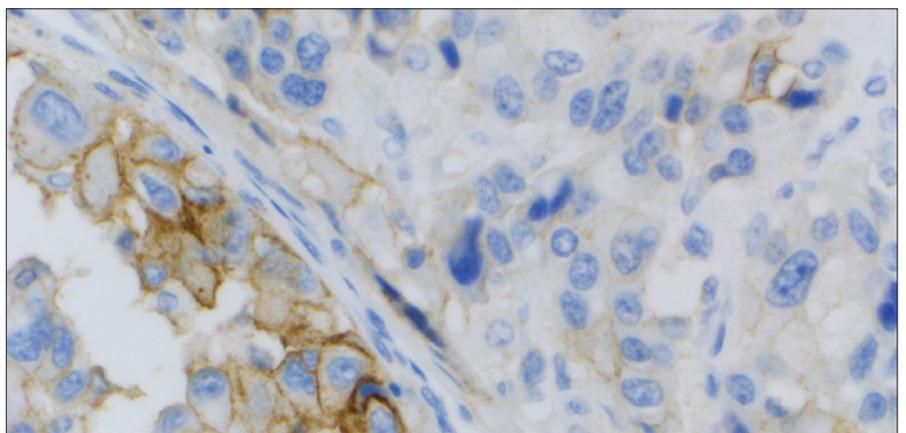


Figure 49c: 40× magnification.

Figure 49a–49c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS 40%.

Challenging Case 8:
TPS 40–60%

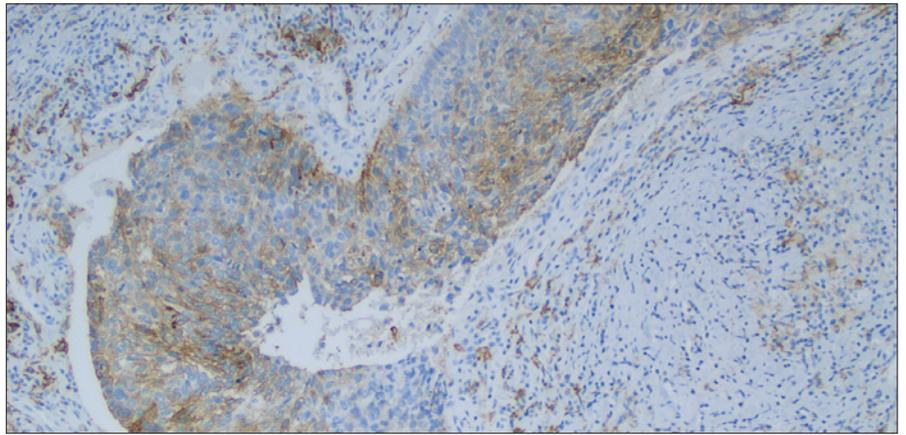


Figure 50a: 10× magnification.

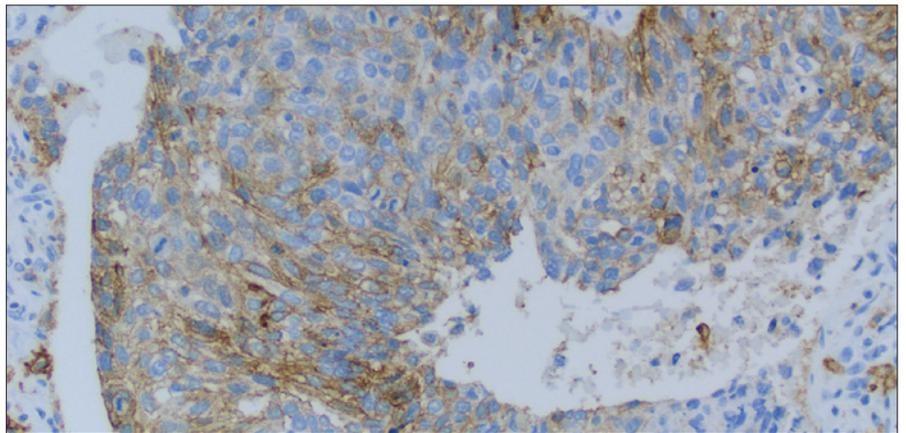


Figure 50b: 20× magnification.

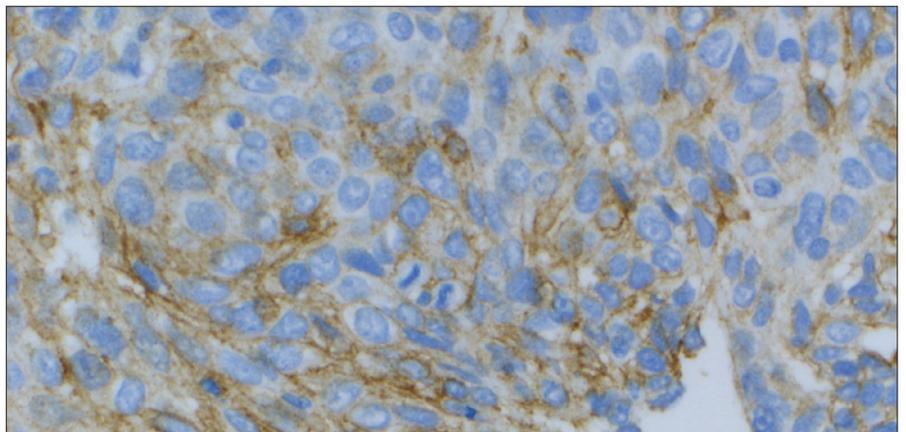


Figure 50c: 40× magnification.

Figure 50a–50c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS 50%.

Challenging Case 9:
TPS 40–60%

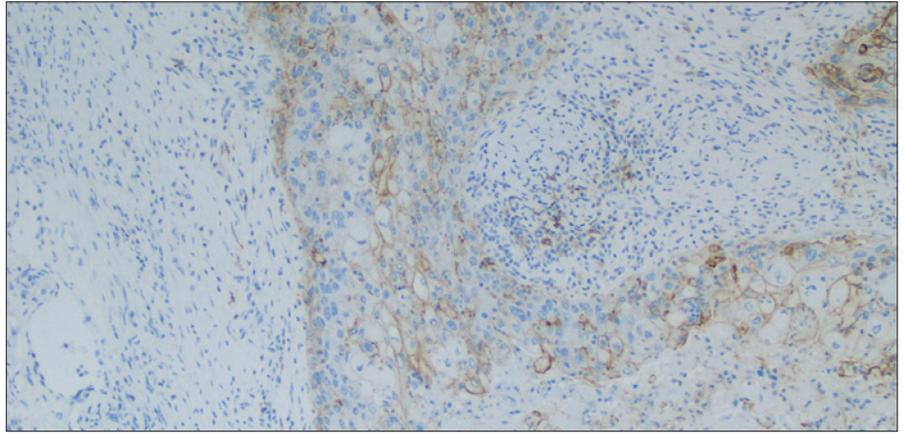


Figure 51a: 10× magnification.

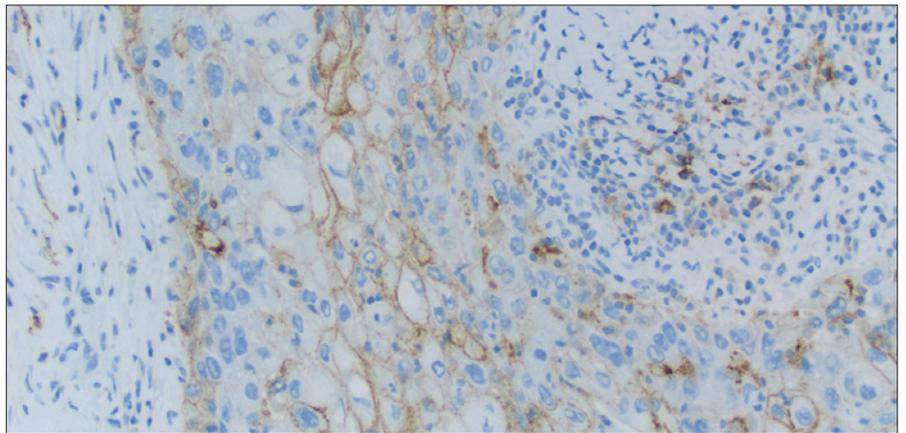


Figure 51b: 20× magnification.

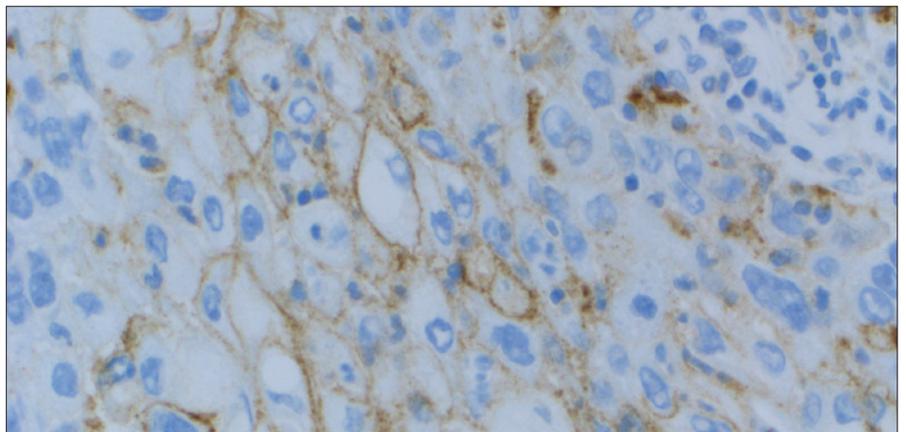


Figure 51c: 40× magnification.

Figure 51a–51c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS 60%.

Challenging Case 10:
TPS 40–60%

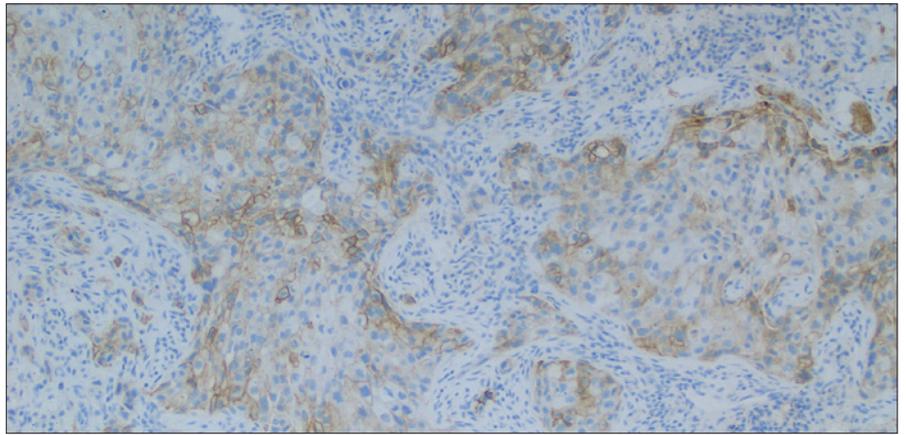


Figure 52a: 10× magnification.

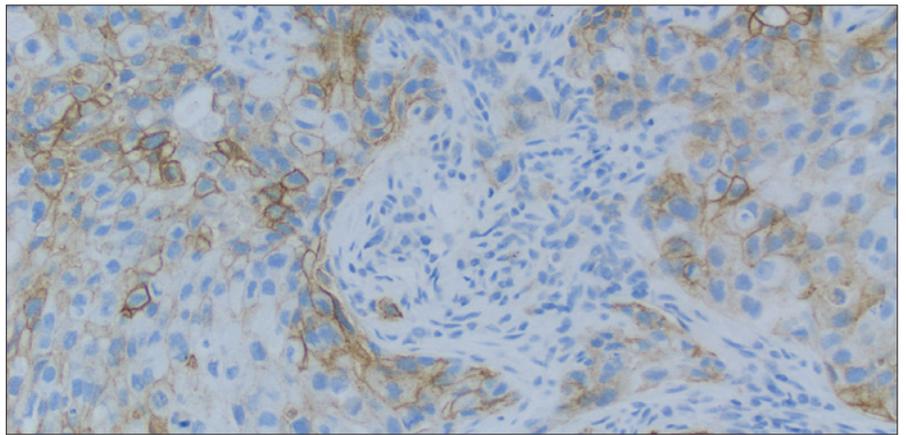


Figure 52b: 20× magnification.

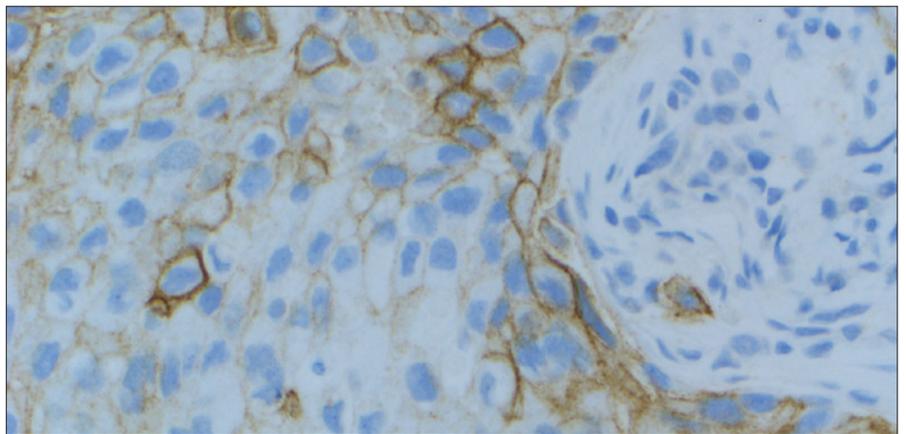


Figure 52c: 40× magnification.

Figure 52a–52c: NSCLC specimen stained with PD-L1 antibody exhibiting TPS 60%.

Artifacts

The following pages provide examples of artifacts you may see when staining with PD-L1 IHC 22C3 pharmDx.

Specific Nonscorable Staining

Specific nonscorable staining is defined as chromogen-related staining due to the anti-PD-L1 antibody/PD-L1 antigen interaction that is present on cells or cellular compartments that are not included when scoring with TPS. It is specific staining because it is present on the PD-L1 slide and absent from the NCR slide; however, it is nonscorable because it is present on either cells (e.g., stromal cells, peripheral nerve, plasma cells, etc.) or cellular compartments (e.g., cytoplasm or nucleus of tumor cells) that are not included in the TPS algorithm. It is recommended that pathologists use their best clinical judgment to determine whether specific nonscorable staining present in the PD-L1 slide interferes with their evaluation of specific scorable PD-L1 expression to such an extent that a confident TPS score cannot be rendered. In cases where interference from specific nonscorable staining prevents confidence in scoring, the PD-L1 slide is considered non-evaluable. Note that even cases with high (> 1+ intensity) specific staining of nonscorable tissue components in the PD-L1 slide are considered evaluable if the pathologist can still provide a confident TPS score from the tissue.

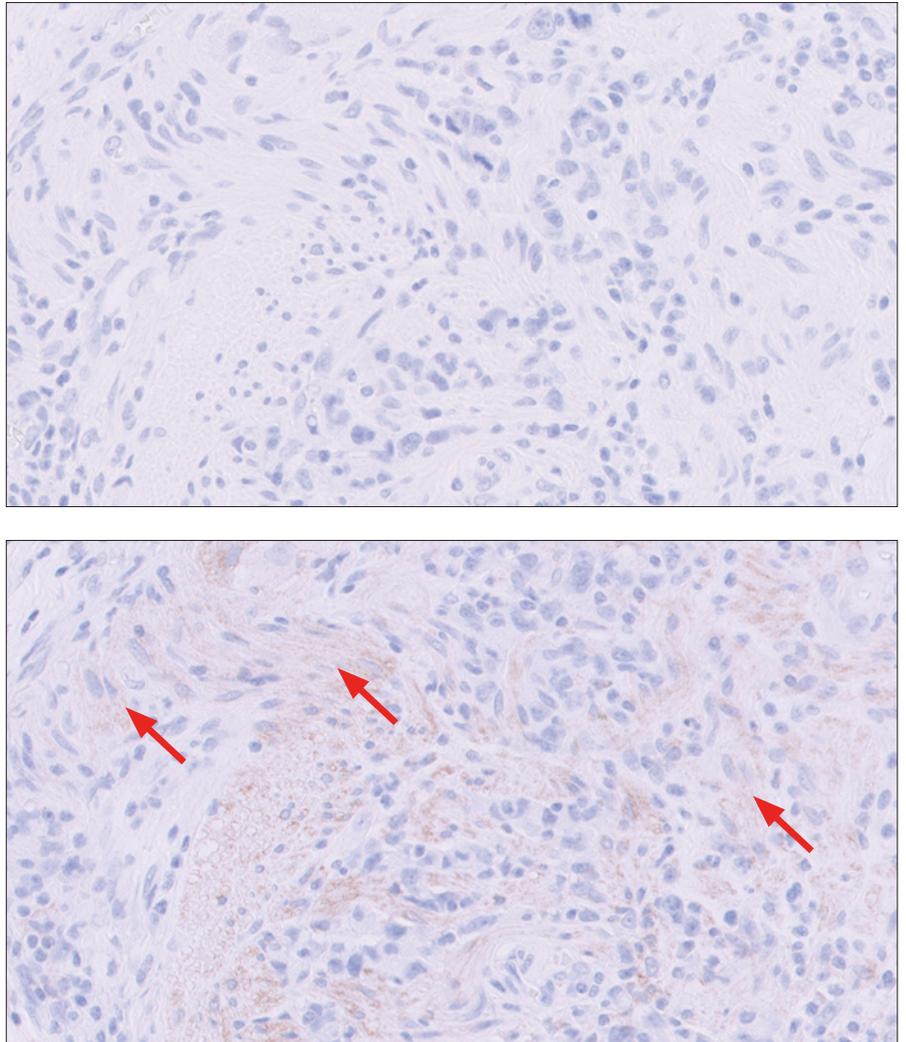


Figure 53a: Gastric or GEJ adenocarcinoma specimen exhibiting $\leq 1+$ intensity specific nonscorable cytoplasmic staining of peripheral nerve cells (arrows). Absence of similar staining in the NCR-stained slide (top) indicates that this cytoplasmic staining in the PD-L1 slide (bottom) is specific, but normal cells such as peripheral nerve cells should be excluded from scoring (20x magnification). Note: Gastric or GEJ adenocarcinoma depicted for demonstration purposes only.

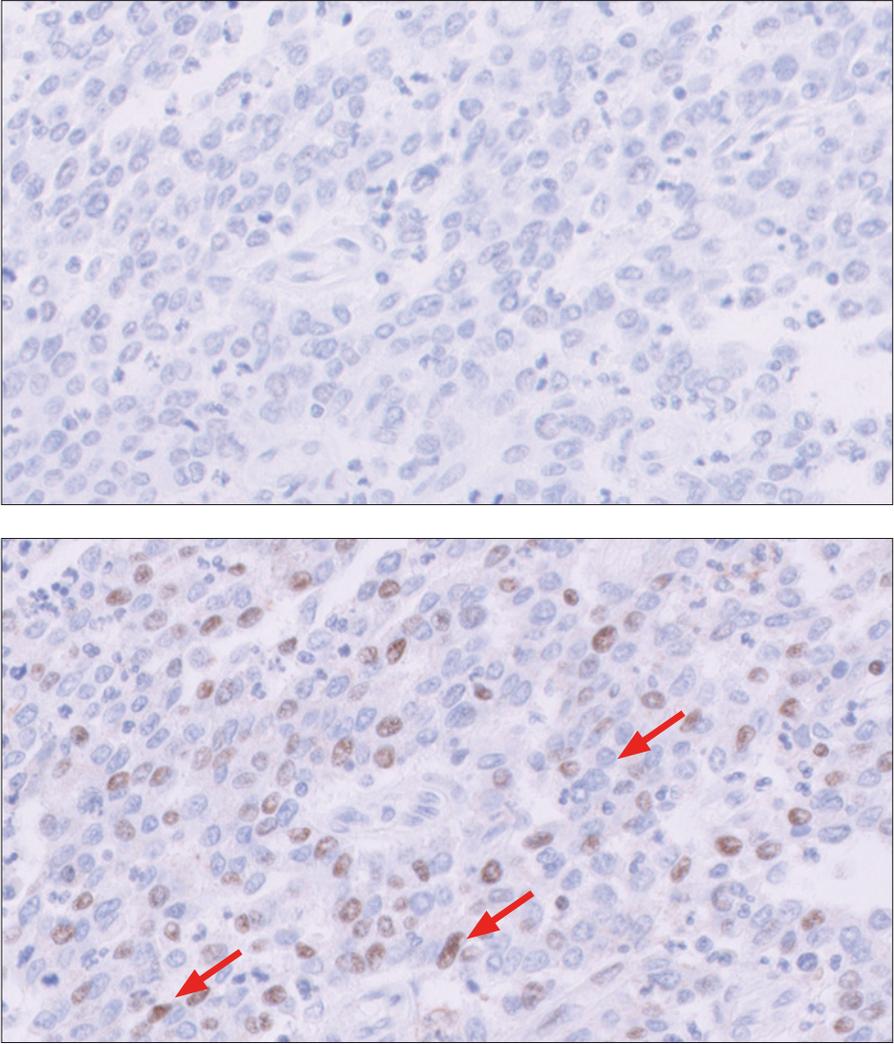


Figure 53b: Gastric or GEJ adenocarcinoma specimen with nuclear staining of tumor cells absent from the NCR-stained slide (top) and present in the PD-L1 stained slide (arrows)(bottom), indicating that this nuclear staining is specific staining. Since the nucleus is a cell compartment that is not scorable, and the nuclear reactivity does not interfere with evaluation of membrane staining on tumor cells, the PD-L1 slide should be considered evaluable (20x magnification).
Note: Gastric or GEJ adenocarcinoma depicted for demonstration purposes only.

Nonspecific Staining

Nonspecific staining is defined as chromogen-related staining that is not related to the anti-PD-L1 antibody/PD-L1 antigen interaction and can be visible either on both the NCR and PD-L1 stained slides, or only on the NCR slide. It is caused by several factors. These factors include, but are not limited to:

- Pre-analytic fixation and processing of the specimen, including use of fixatives other than neutral buffered formalin (not recommended)
- Incomplete removal of paraffin from the section
- Incomplete rinsing of reagents from slides during staining
- Drying of slides; ensure slides remain wet with buffer while loading onto Autostainer Link 48, prior to initiating run and during the staining procedure
- Cross-reactivity of the secondary antibody in the detection system
- Reagent trapping (tissue folding; tissue drying; hydrophobic or ionic interactions with 'sticky' tissues or substances such as cartilage, muscle fibers, dense fibrosis, mucin, necrotic debris)

The nonspecific staining of the NCR-stained test section is useful in determining the level of nonspecific staining in the PD-L1 stained test section.

All specimens must have $\leq 1+$ nonspecific staining to be considered acceptable for PD-L1 expression evaluation.

When staining of cell nuclei is present in both the specimen slide stained with the PD-L1 primary antibody as well as in the NCR-stained slide, or only in the NCR-stained slide, it is considered nonspecific nuclear staining and, if present, should be $\leq 1+$ intensity within the scorable tumor region(s) of both the PD-L1 and NCR-stained slides and excluded from scoring. If the intensity of the nonspecific nuclear staining within scorable tumor region(s) is $> 1+$ in the NCR-stained slide, or both the NCR- and PD-L1 stained slide, the PD-L1 slide should be marked as non-evaluable and a retest of the specimen should be performed.

Nonspecific staining with PD-L1 IHC 22C3 pharmDx is rare.

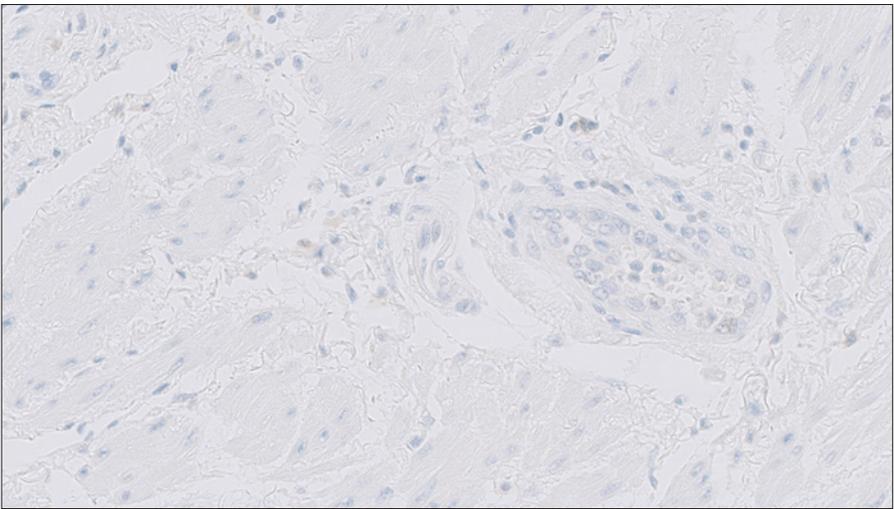
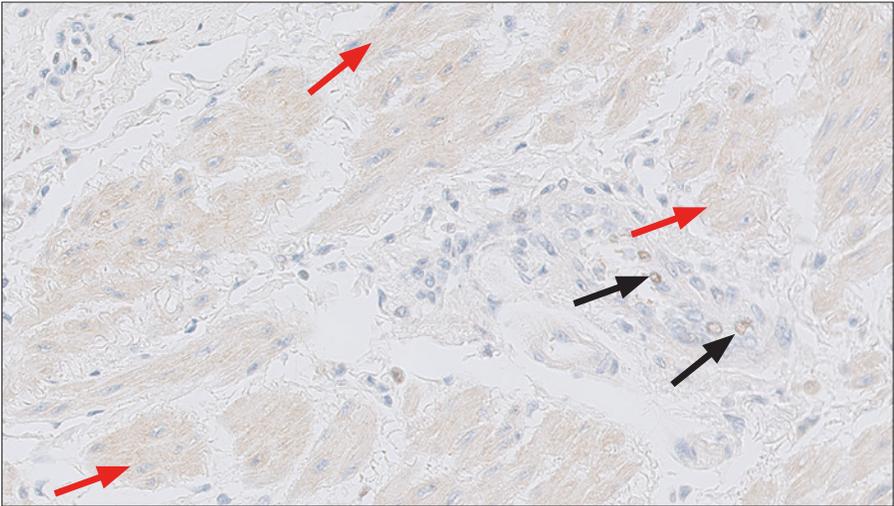


Figure 54a: Esophageal adenocarcinoma specimen stained with NCR (top) exhibiting acceptable ($\leq 1+$ intensity) nonspecific staining on smooth muscle cells (red arrows) and occasional endothelial cell nuclei (black arrows). Note that in this case, the nonspecific staining is not also present in the PD-L1 slide (bottom) (20x magnification).

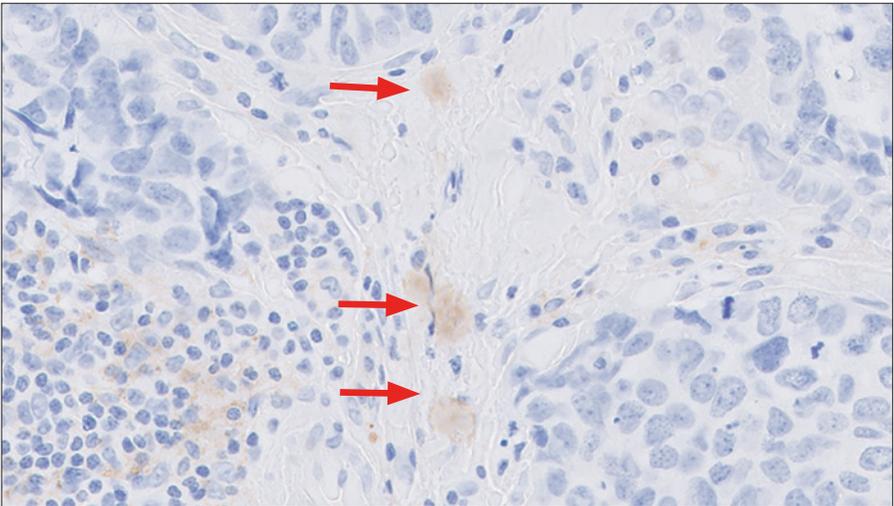


Figure 54b: Negative Control Reagent (NCR) exhibiting nonspecific staining in TNBC (arrows) (20x magnification).

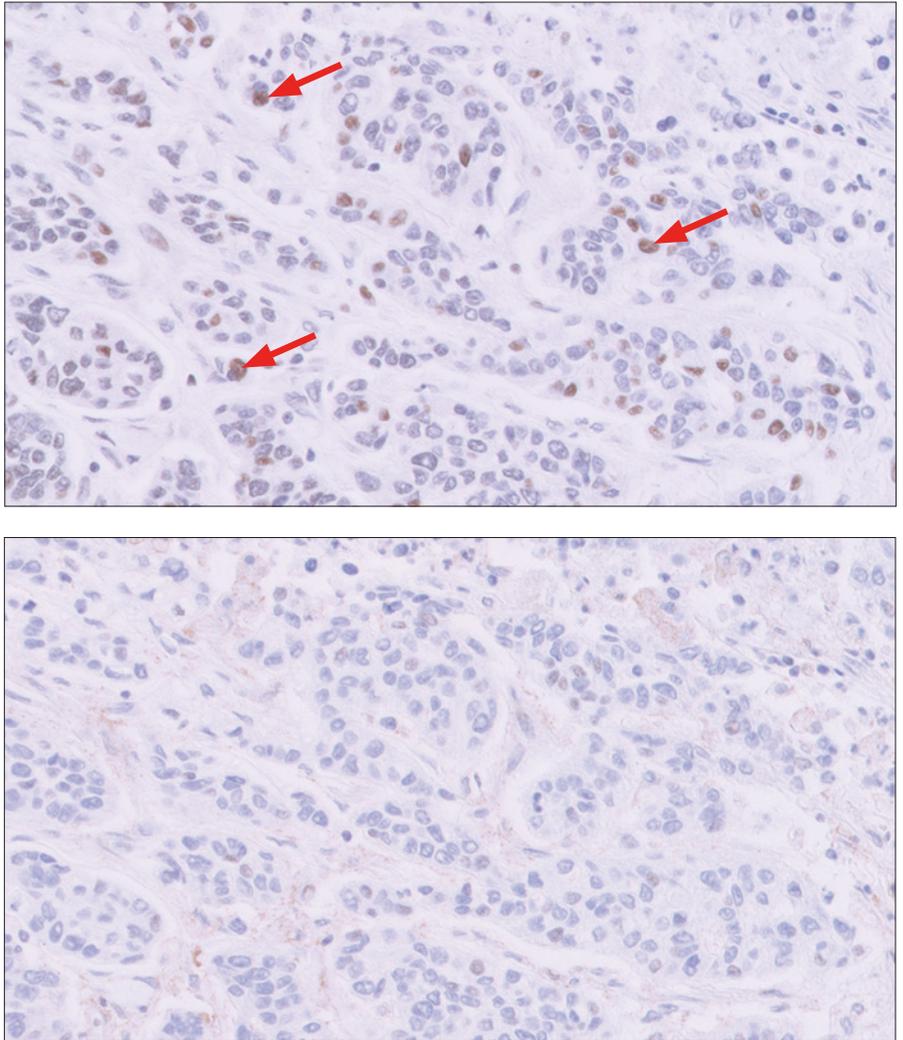


Figure 54c: Gastric or GEJ adenocarcinoma specimen exhibiting nonspecific nuclear staining (arrows) that is present in both the NCR-(top) and PD-L1 (bottom) stained slides, but is stronger ($\geq 1+$) intensity in the NCR slide. As the acceptance criteria for nonspecific staining, including nuclear staining, in the NCR and PD-L1 slides or NCR slide alone is $\leq 1+$ intensity, the PD-L1 stained slide in this case should be considered non-evaluable (20x magnification).

Key Point

All specimens must have $\leq 1+$ nonspecific staining, including nuclear staining

Edge Artifact

Commonly, edge artifact is linked to the following pre-analytic factors:

- Thick tissue sections
- Drying of tissue prior to fixation or during staining procedure

Both factors can lead to accentuation of staining at the periphery of the section and minimal staining or nonstaining in the central portion. Only PD-L1 staining at the edge of the tissue section is excluded from scoring.

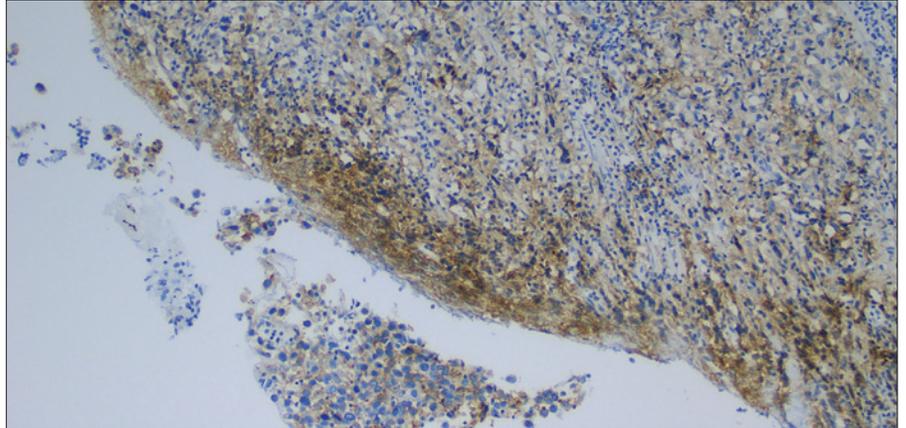


Figure 55a: NSCLC specimen stained with PD-L1 primary antibody exhibiting edge artifact staining; edge staining should be excluded from the scoring (4× magnification).

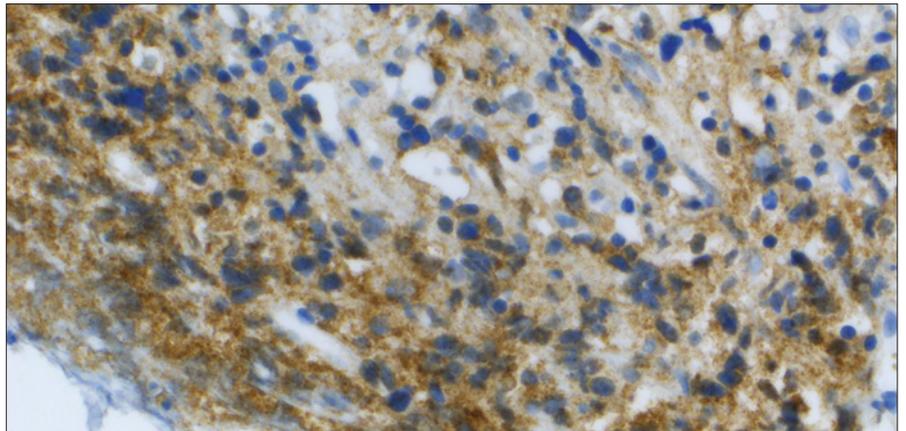


Figure 55b: NSCLC specimen stained with PD-L1 primary antibody exhibiting edge artifact staining; edge staining should be excluded from the scoring (20× magnification).

Key Point

Scoring of the edge of a specimen should be avoided if staining is inconsistent with the rest of the specimen

Crush Artifact

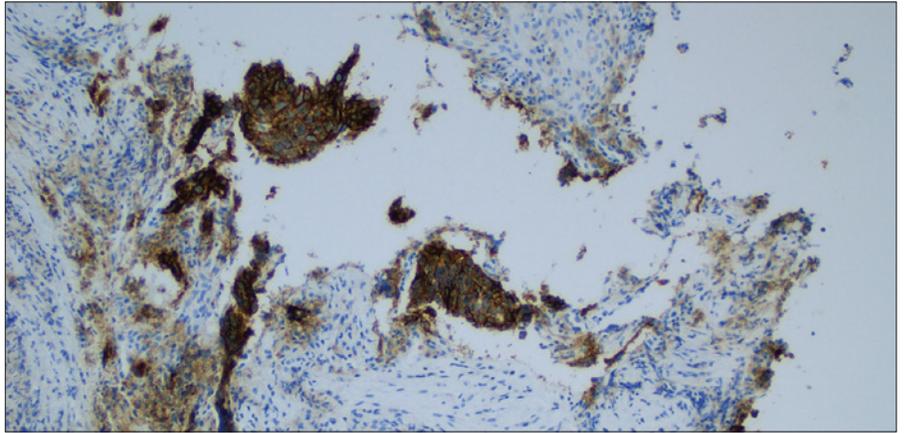


Figure 56: NSCLC specimen stained with PD-L1 primary antibody exhibiting crush artifact (10× magnification).

Areas of the examined section exhibiting cytologically and morphologically distorted secondary crush artifact may show exaggerated staining and should be excluded from the score.

Key Point

Scoring of crush artifact should be avoided

Necrosis

Necrosis can be described as morphological changes indicative of cell death with undefined cellular detail. Necrosis is often present in NSCLC specimens and should be excluded from scoring.

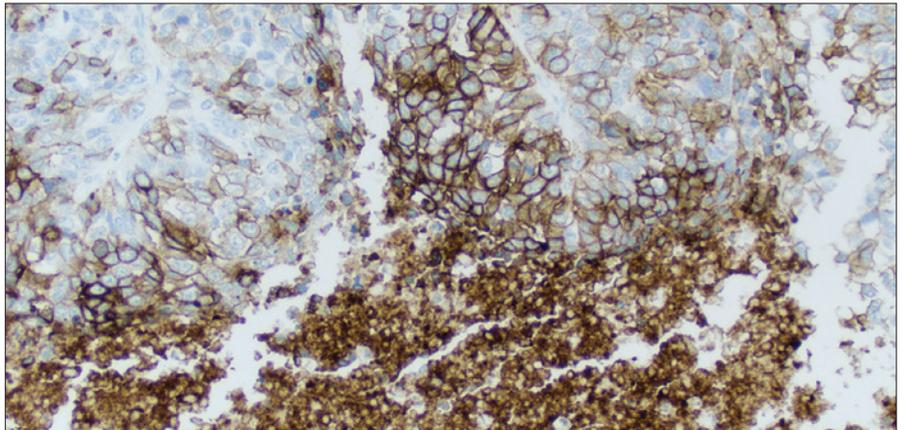


Figure 57: NSCLC specimen stained with PD-L1 primary antibody exhibiting strong staining of necrosis and viable tumor cells; necrosis staining should be excluded from the scoring (20× magnification).

Key Point

Scoring of necrotic areas should be excluded from the TPS

Poor Fixation

Standardization of fixation is very important when using PD-L1 IHC 22C3 pharmDx. Sub-optimal fixation on tissues may give erroneous results.

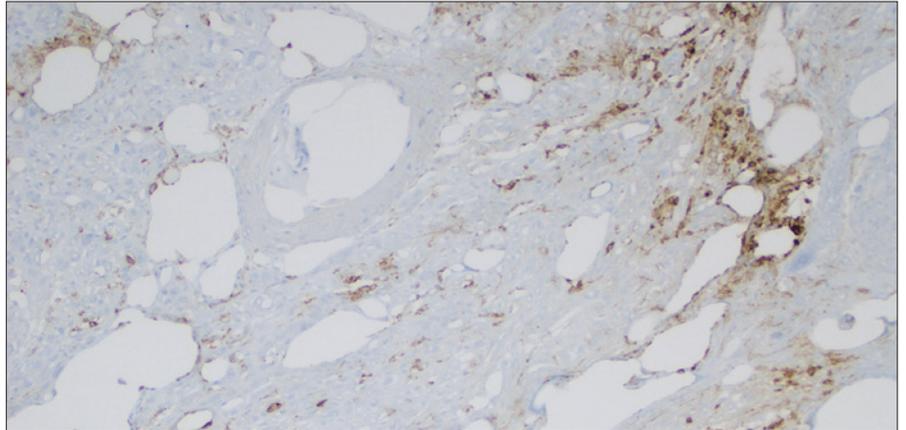


Figure 58: NSCLC specimen stained with PD-L1 primary antibody exhibiting poor tissue fixation (10× magnification).

Key Point

Proper fixation is important for accurate diagnosis

Control Cell Line (CCL) Appendix

Passing CCL

Passing PD-L1 Negative CCL

- No cells with membrane staining*
- Nonspecific staining < 1+ intensity*

* Note that staining of a few cells in the MCF-7 cell pellet may occasionally be observed. The following acceptance criteria are applicable: the presence of ≤ 10 total cells with distinct cell membrane staining and/or nonspecific staining with $\geq 1+$ intensity within the boundaries of the MCF-7 cell pellet are acceptable.

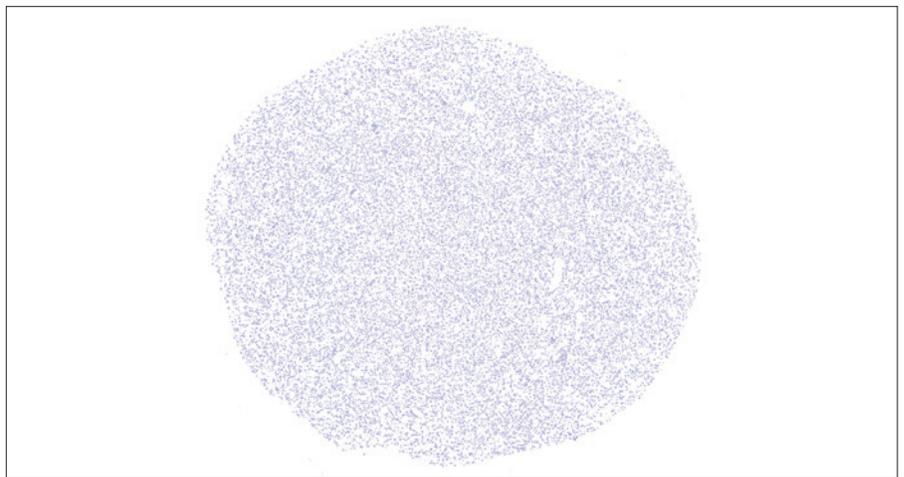


Figure 59: Ideal MCF-7 cell pellet (2 \times magnification).

Passing PD-L1 Positive CCL

- Cell membrane staining of $\geq 70\%$ of cells
- $\geq 2+$ average staining intensity of cells with membrane staining
- Nonspecific staining is < 1+ intensity



Figure 60: Ideal NCI-H226 cell pellet (2 \times magnification).

Borderline Passing CCL

Borderline Passing vs. Passing PD-L1 Positive CCL

Borderline Passing PD-L1 positive CCL

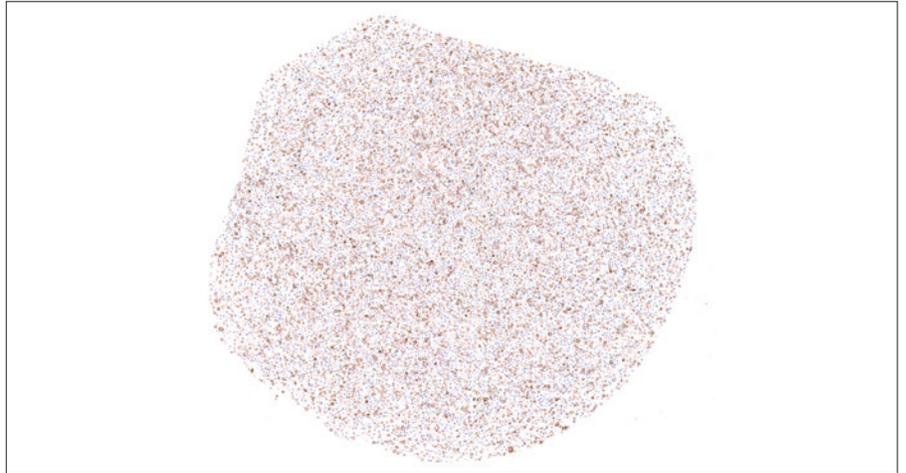


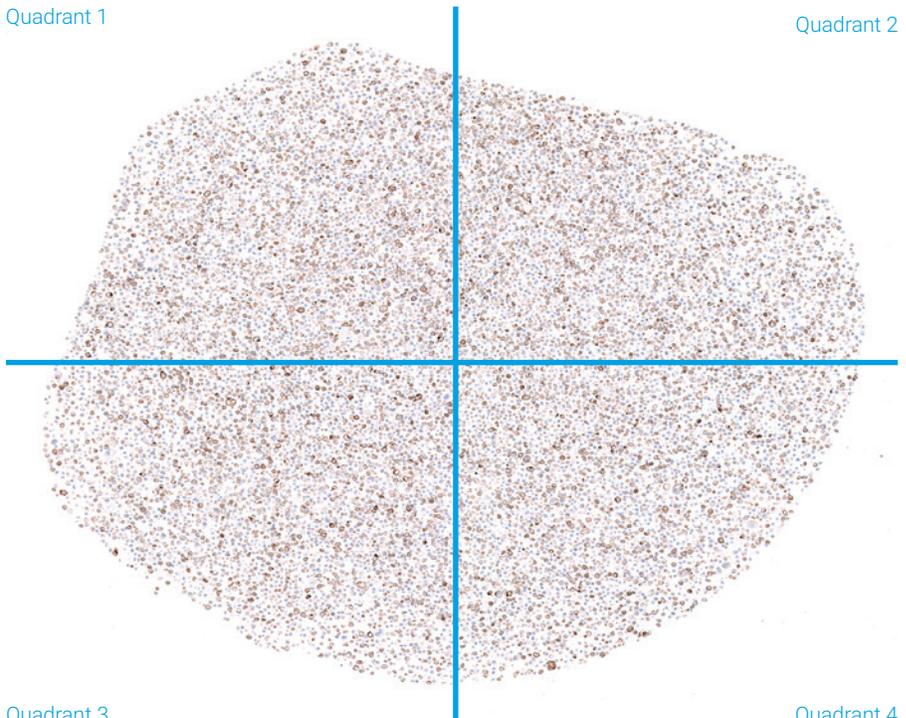
Figure 61: NCI-H226 cell pellet (2× magnification).

Evaluation Strategy for Borderline Passing PD-L1 Positive CCL

For a borderline PD-L1 positive CCL, to determine the total percentage of cells staining in the cell pellet and the average staining intensity of all staining cells in the pellet, the cell pellet can be split into quadrants and inspected at 20× magnification.

Quadrant 1

Quadrant 2



Quadrant 3

Quadrant 4

Quadrant 1

In Quadrant 1 approximately 70% of cells exhibit membrane staining, and the average staining intensity of all staining cells in this quadrant is $\geq 2+$.

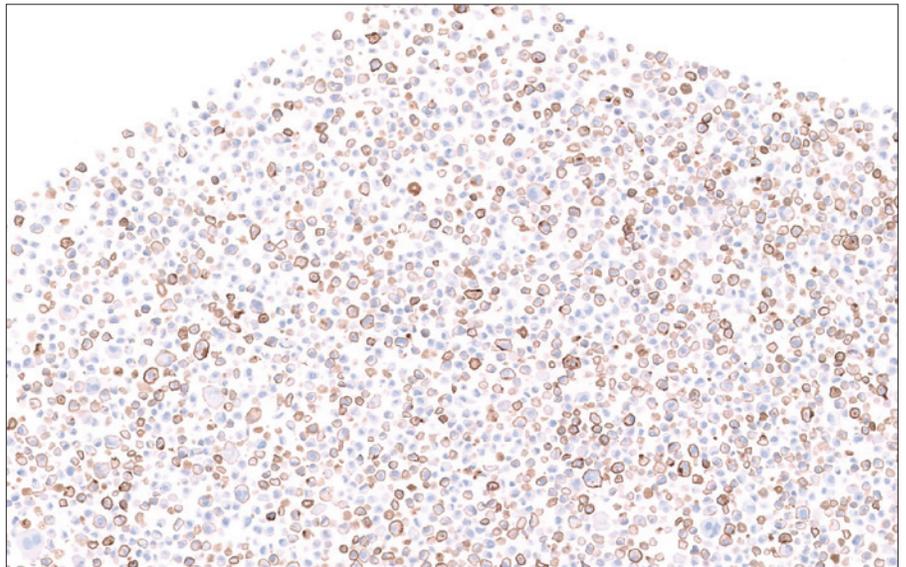


Figure 62: NCI-H226 cell pellet (5 \times magnification).

Quadrant 2

In Quadrant 2 approximately 75% of cells exhibit membrane staining, and the average staining intensity of all staining cells in this quadrant is $\geq 2+$.

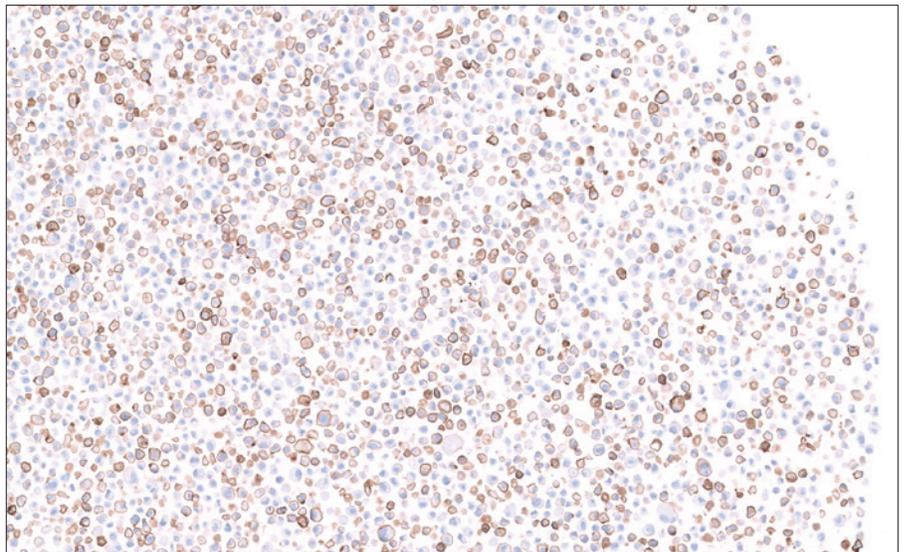


Figure 63: NCI-H226 cell pellet (5 \times magnification).

Quadrant 3

In Quadrant 3 approximately 70% of cells exhibit membrane staining, and the average staining intensity of all staining cells in this quadrant is $\geq 2+$.

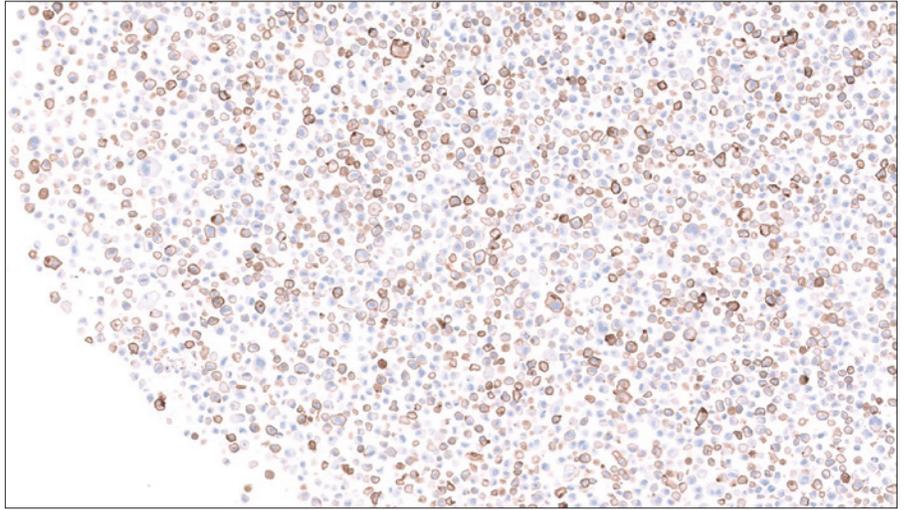


Figure 64: NCI-H226 cell pellet (5× magnification).

Quadrant 4

In Quadrant 4 approximately 65% of cells exhibit membrane staining, and the average staining intensity of all staining cells in this quadrant is $\geq 2+$.

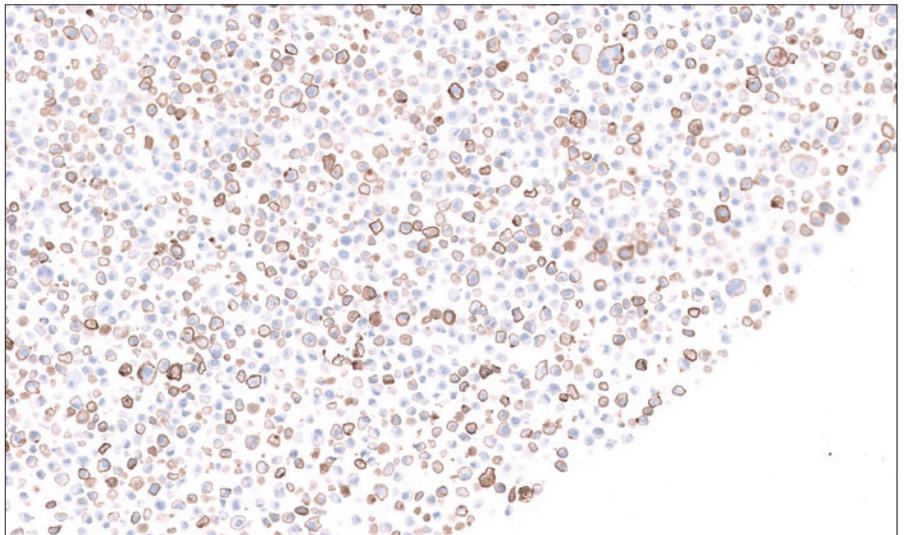
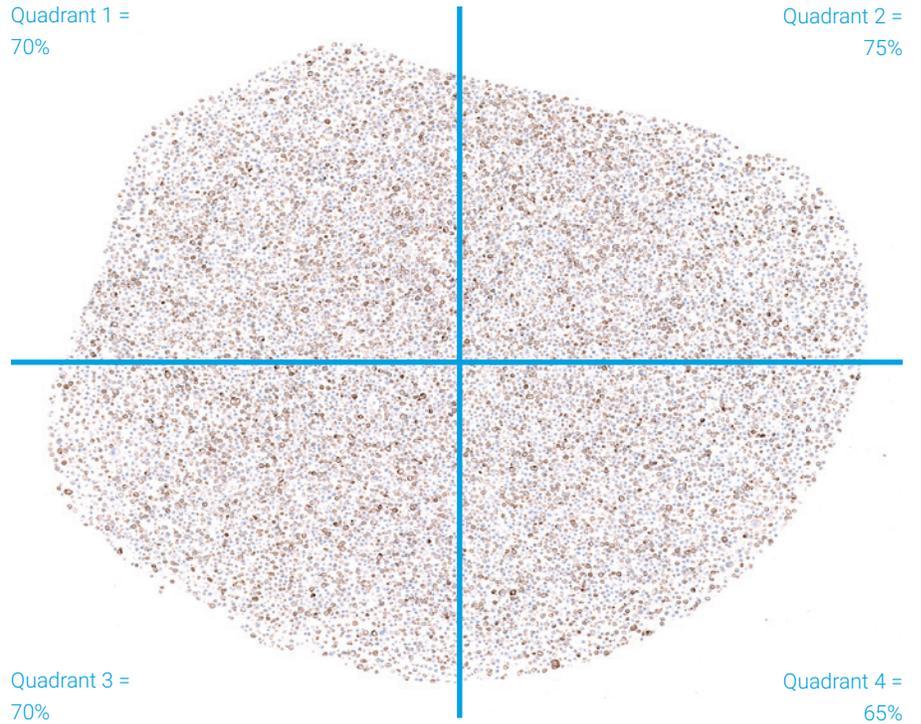


Figure 65: NCI-H226 cell pellet (5× magnification).

Calculation

1. Calculate the average percentage of cells exhibiting membrane staining across all 4 quadrants to estimate the total percentage of cells exhibiting membrane staining across the entire PD-L1 positive CCL pellet
2. Determine whether the average staining intensity across all cells with membrane staining in the pellet is $\geq 2+$ intensity



$$\frac{70 + 75 + 70 + 65}{4} = 70$$

- The overall percentage of cells with membrane staining = 70%
- The average staining intensity of all cells with membrane staining in the cell pellet is $\geq 2+$

NCI-H226 positive control cell pellet meets acceptance criteria.

Failed CCL

Example 1: Passing PD-L1 Negative CCL with Failed PD-L1 Positive CCL

Passing PD-L1 negative CCL

- No cells with membrane staining.*
- Nonspecific staining < 1+ intensity.*

* Note that staining of a few cells in the MCF-7 cell pellet may occasionally be observed. The following acceptance criteria are applicable: the presence of ≤ 10 total cells with distinct cell membrane staining and/or nonspecific staining with $\geq 1+$ intensity within the boundaries of the MCF-7 cell pellet are acceptable.

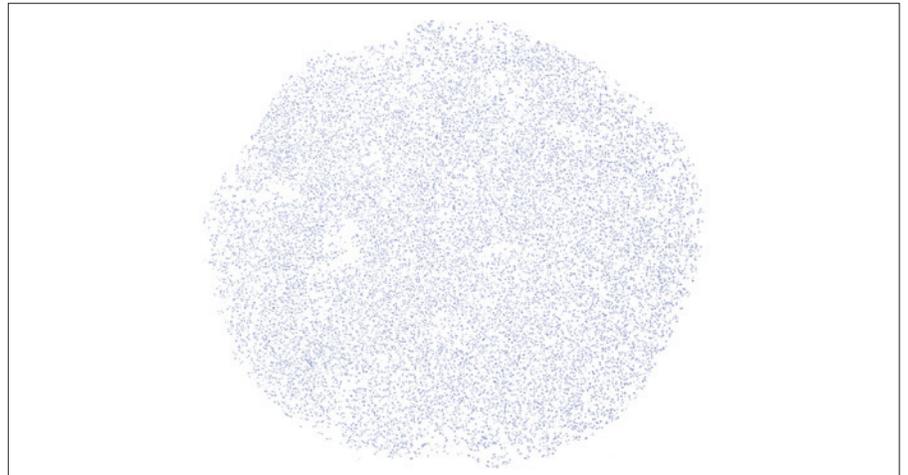


Figure 66: MCF-7 cell pellet (2x magnification).

Failed PD-L1 positive CCL

- Less than 70% of cells exhibit membrane staining, and the average staining intensity across all cells with membrane staining in the pellet is < 2+

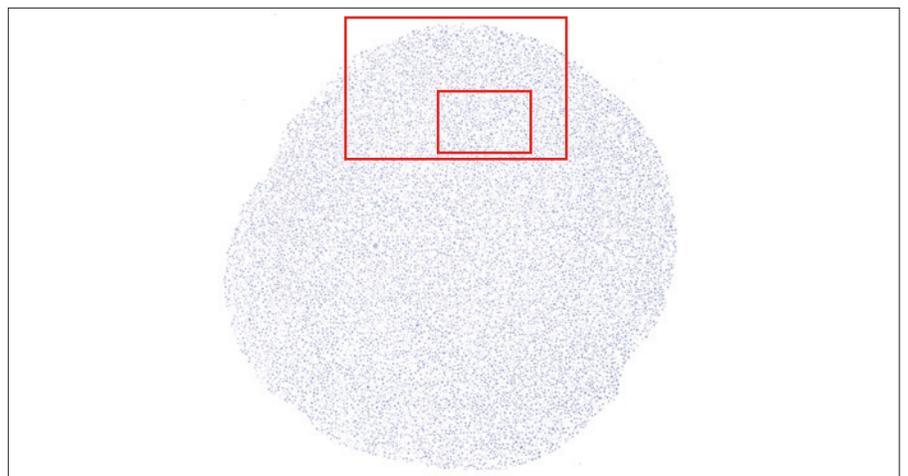


Figure 67: NCI-H226 cell pellet (2x magnification).

See following images for higher magnification images depicting details of failure.

Failed PD-L1 positive CCL (10×)

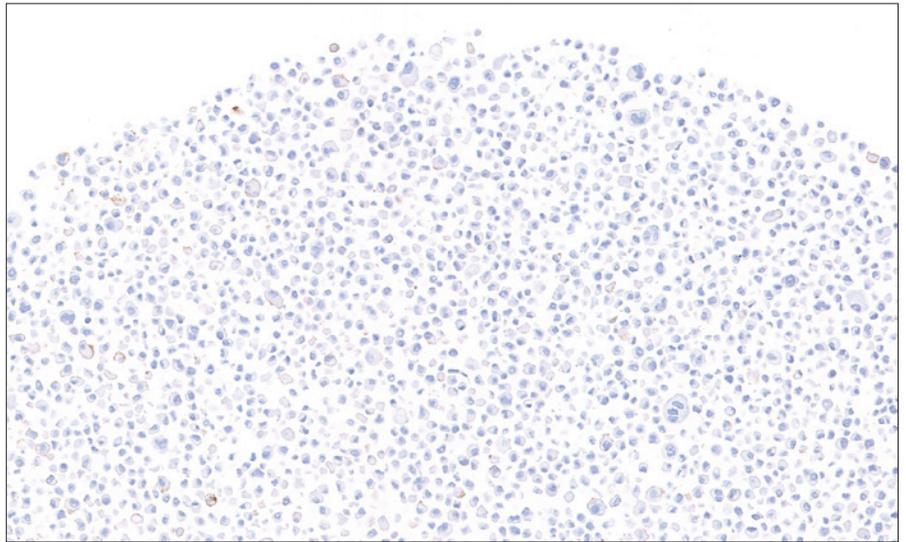


Figure 68: NCI-H226 cell pellet (10× magnification).

Failed PD-L1 positive CCL (20×)

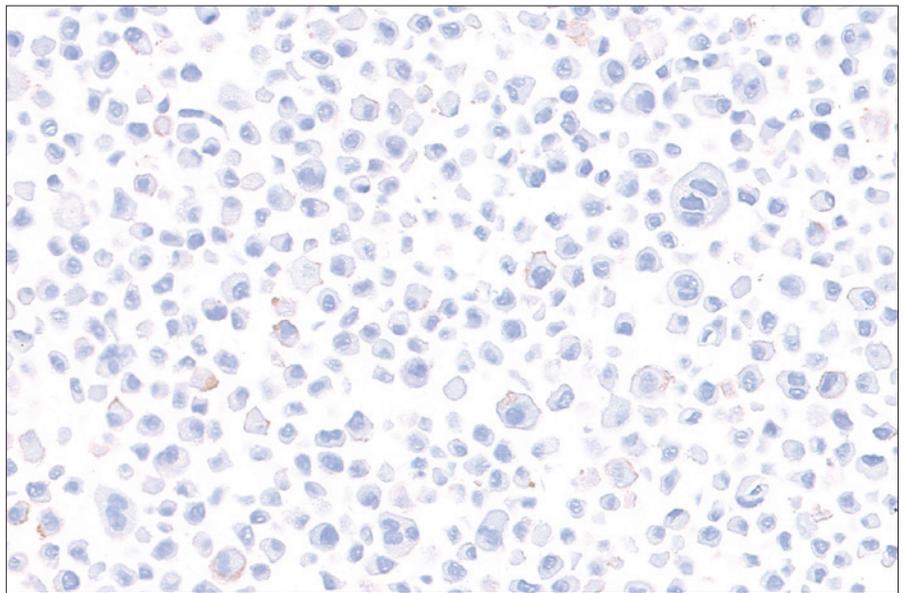


Figure 69: NCI-H226 cell pellet (20× magnification).

Example 2: Passing PD-L1 Negative CCL with Failed PD-L1 Positive CCL

Passing PD-L1 negative CCL

- No cells with membrane staining.*
- Nonspecific staining < 1+ intensity.*

* Note that staining of a few cells in the MCF-7 cell pellet may occasionally be observed. The following acceptance criteria are applicable: the presence of ≤ 10 total cells with distinct cell membrane staining and/or nonspecific staining with $\geq 1+$ intensity within the boundaries of the MCF-7 cell pellet are acceptable.

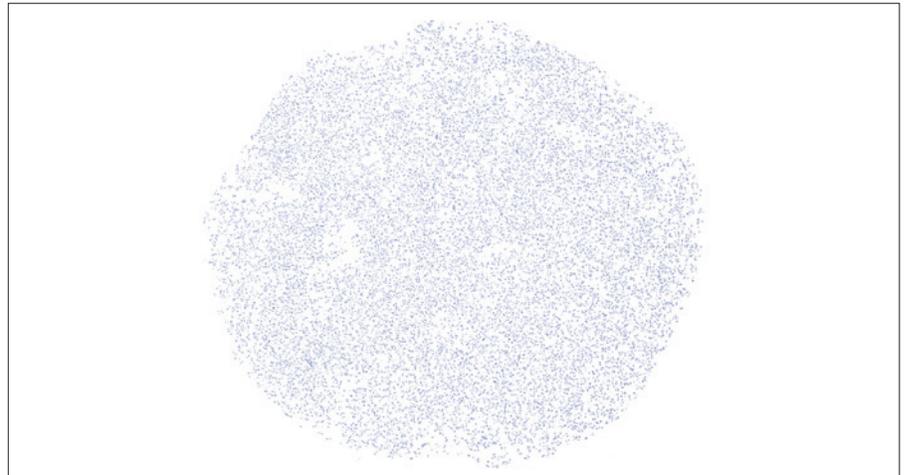


Figure 70: MCF-7 cell pellet (2x magnification).

Failed PD-L1 positive CCL

- Less than 70% of cells exhibit membrane staining, and the average staining intensity across all cells with membrane staining in the pellet is < 2+

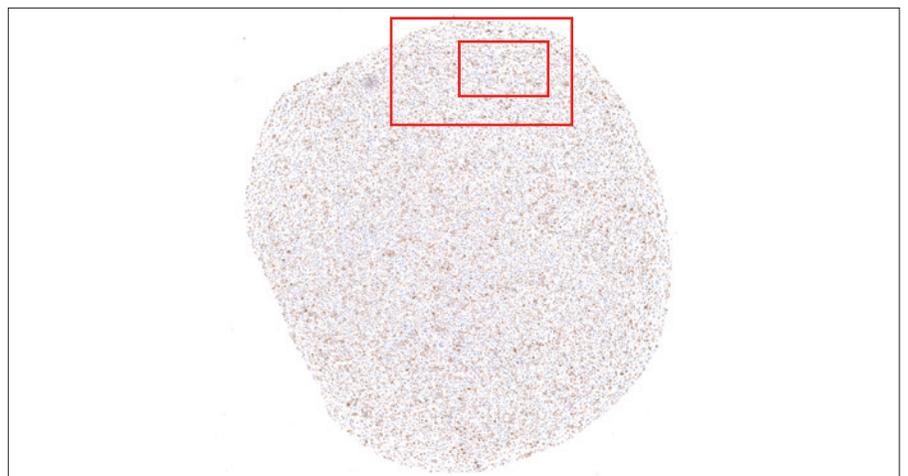


Figure 71: NCI-H226 cell pellet (2x magnification).

See following images for higher magnification images depicting details of failure.

Failed PD-L1 positive CCL (10×)

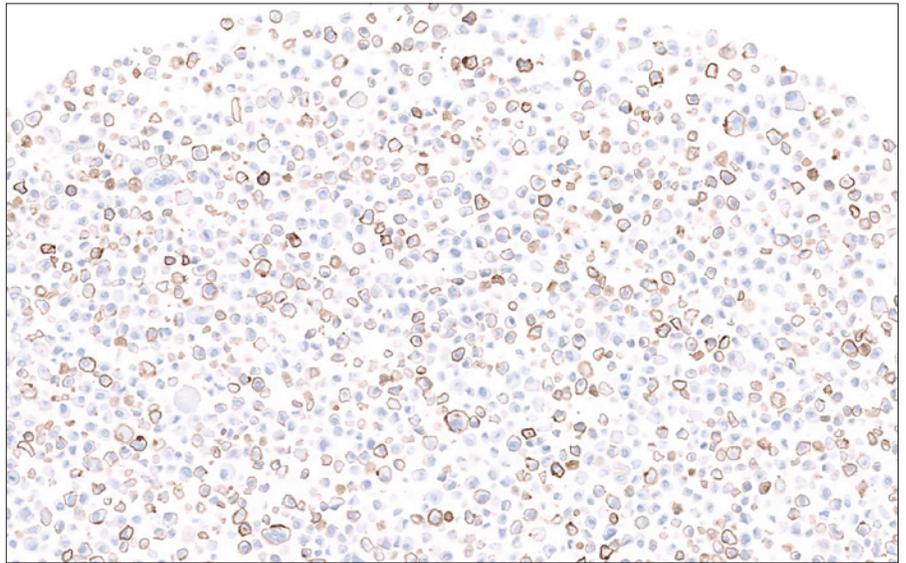


Figure 72: NCI-H226 cell pellet (10× magnification).

Failed PD-L1 positive CCL (20×)

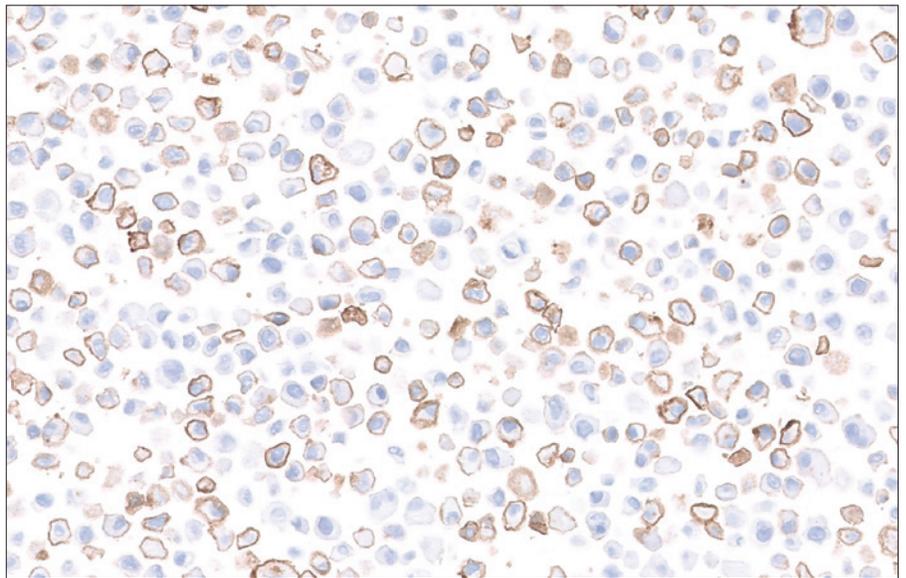


Figure 73: NCI-H226 cell pellet (20× magnification).

Example 3: Passing PD-L1 Positive CCL with Failed PD-L1 Negative CCL

Passing PD-L1 positive CCL

- Cell membrane staining of $\geq 70\%$ of cells
- $\geq 2+$ average staining intensity of cells with membrane staining
- Nonspecific staining is $< 1+$

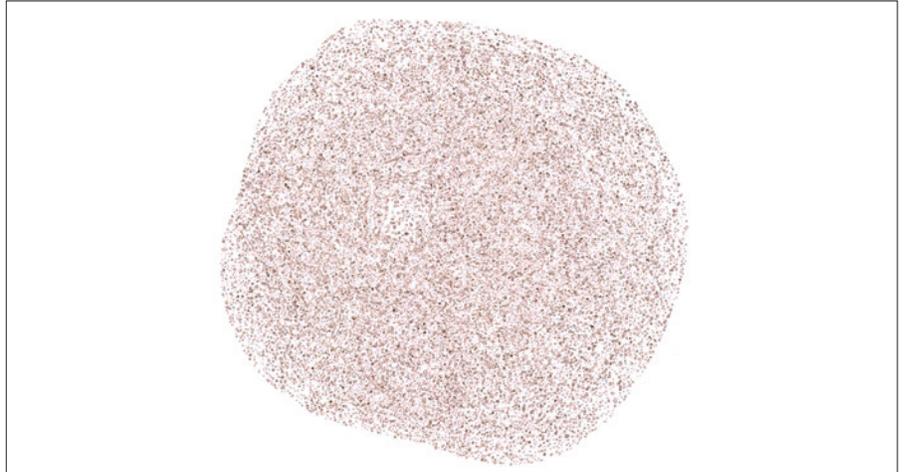


Figure 74: NCI-H226 cell pellet (2x magnification).

Failed PD-L1 negative CCL

- Nonspecific (nuclear) staining is $\geq 1+$ staining intensity
- There are > 10 total cells with distinct cell membrane or cytoplasmic staining that is $\geq 1+$ intensity

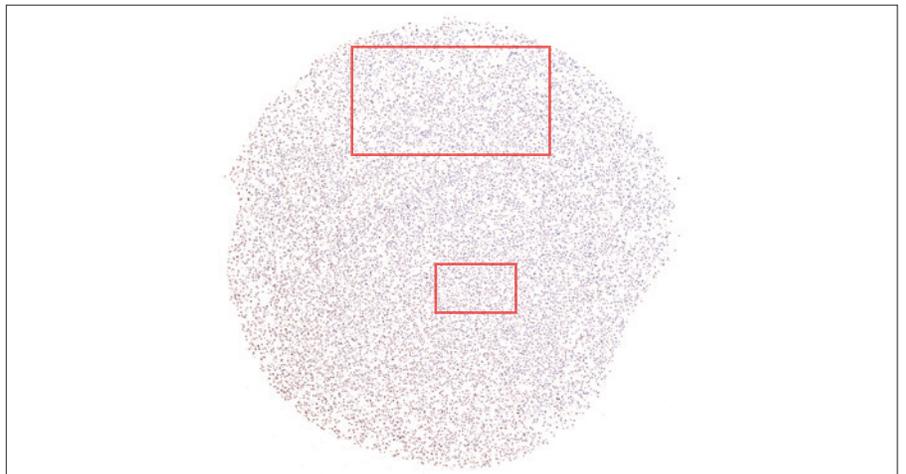


Figure 75: MCF-7 cell pellet (2x magnification).

See following images for higher magnification images depicting details of failure.

Failed PD-L1 negative CCL (10×)

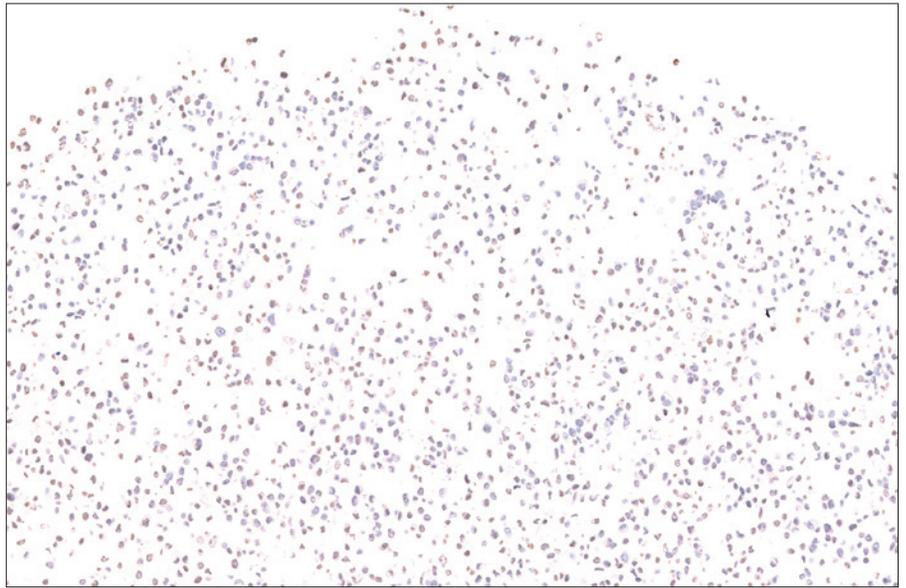


Figure 76: MCF-7 cell pellet (10× magnification).

Failed PD-L1 negative CCL (20×)

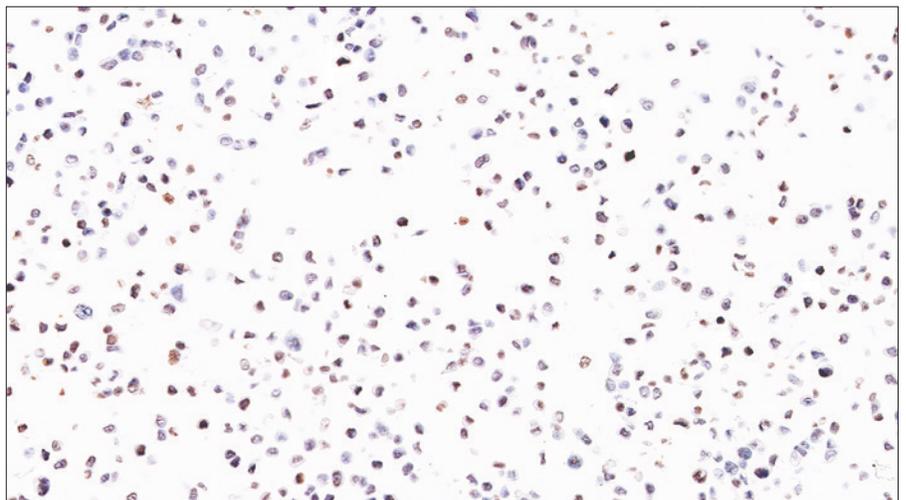


Figure 77: MCF-7 cell pellet (20× magnification).

Troubleshooting Guide

Troubleshooting Guidelines for PD-L1 IHC 22C3 pharmDx

For further troubleshooting help, contact your local Agilent representative.

Problem	Probable Cause	Suggested Action
No staining of slides	Programming error	Verify that the PD-L1 IHC 22C3 pharmDx program was selected for programming of slides
	Lack of reaction with DAB+ Substrate-Chromogen Solution (DAB)	Verify that DAB+ Substrate-Chromogen Solution was prepared properly
	Sodium azide in wash buffer	Use only Dako Wash Buffer (Code K8007)
	Degradation of Control Slide	Check kit expiration date and kit storage conditions on outside of package
Weak staining of specimen slides	Inappropriate fixation method used	Ensure that only neutral buffered formalin fixative and approved fixation methods are used
	Insufficient reagent volume applied	Check size of tissue section and reagent volume applied
	Inappropriate wash buffer used	Use only Dako Wash Buffer (Code K8007)
Weak staining of specimen slides or of the positive cell line on the Control Cell Line Slide provided with the kit	Inadequate target retrieval	Verify that the 3-in-1 pretreatment procedure was correctly performed
	Inappropriate wash buffer used	Use only Dako Wash Buffer (Code K8007)
Excessive nonspecific staining of slides	Paraffin incompletely removed	Verify that the 3-in-1 pretreatment procedure was correctly performed
	Slides dried after the 3-in-1 pretreatment procedure was performed	Ensure slides remain wet with 1× EnVision FLEX Wash Buffer after the 3-in-1 pretreatment procedure and after loading on the Autostainer Link 48. Ensure that the Autostainer Link 48 lid is properly closed to prevent reagent evaporation during the staining procedure.
	Nonspecific binding of reagents to tissue section	Check for proper fixation of the specimen and/or the presence of necrosis
	Inappropriate fixation method used	Ensure that only neutral buffered formalin fixative and recommended fixation methods are used
	Warped Autostainer Link 48 slide racks used	Ensure that only level Autostainer Link 48 slide racks are used
Tissue detached from slides	Use of incorrect microscope slides	Use Dako FLEX IHC Microscope Slides, (Code K8020), or Superfrost Plus slides
	Inadequate preparation of specimens	Cut sections should be placed in a 58 ± 2 °C oven for 1 hour prior to staining
Excessively strong specific staining	Inappropriate fixation method used	Ensure that only approved fixatives and fixation methods are used
	Inappropriate wash buffer used	Use only Dako Wash Buffer (Code K8007)
Target Retrieval Solution is cloudy in appearance when heated	When heated, the Target Retrieval Solution turns cloudy in appearance	This is normal and does not influence staining

Continued on next page

Problem	Probable Cause	Suggested Action
1× Target Retrieval Solution does not meet pH specifications	pH meter is not calibrated correctly	Ensure pH meter is calibrated per manufacturer’s recommendations. After re-calibration, re-test the pH of 1× Target Retrieval Solution. Do not modify the pH of 1× Target Retrieval Solution. If the pH is outside the acceptable range (6.1 ± 0.2), discard 1× Target Retrieval Solution. Prepare new 1× Target Retrieval Solution. Check the pH of the new 1× Target Retrieval Solution
	Inferior quality water is used to dilute the Target Retrieval Solution concentrate	Ensure that distilled or deionized water is used to prepare 1× Target Retrieval Solution
	Incorrect Target Retrieval Solution is used	Ensure that the correct Target Retrieval Solution specified in “Materials Required but not Supplied” and/or “Reagent Preparation” section(s) is used
Nonspecific > 1+ nuclear staining on PD-L1 and/or NCR slides	Specimen slides dried prior to initiating the Autostainer Link 48 staining procedure	Ensure slides remain wet with 1x Envision FLEX Wash Buffer after Deparaffinization, Rehydration, and Target Retrieval (3-in-1) procedure and after loading on the Autostainer Link 48. Ensure that the Autostainer Link 48 lid is properly closed to prevent reagent evaporation during the staining procedure.
	Improper manual slide rinsing with 1x EnVision FLEX Wash Buffer before loading on the Autostainer Link 48	Slide racks should be placed one rack at a time on the Autostainer Link 48 and then 1x EnVision FLEX Wash Buffer should be manually applied to the slides using a wash bottle. Ensure slides remain wet prior to initiating the Autostainer Link 48 procedure.

Note: If the problem cannot be attributed to any of the above causes, or if the suggested corrective action fails to resolve the problem, please call Agilent Pathology Support for further assistance. Additional information on staining techniques and specimen preparation can be found in the Education Guide: Immunohistochemical Staining Methods (available from Agilent Technologies).

Clinical Performance Evaluation

KEYTRUDA® (pembrolizumab)

KN042: First-line Treatment of Metastatic NSCLC as a Single Agent

The efficacy of KEYTRUDA was investigated in KEYNOTE-042 (NCT02220894), a randomized, multicenter, open-label, active-controlled trial conducted in 1274 patients with stage III NSCLC, who were not candidates for surgical resection or definitive chemoradiation, or metastatic NSCLC, whose tumors expressed PD-L1 (TPS \geq 1%) by an immunohistochemistry assay using PD-L1 IHC 22C3 pharmDx, and who had not received prior systemic treatment for metastatic NSCLC. Patients with EGFR or ALK genomic tumor aberrations; autoimmune disease that required systemic therapy within 2 years of treatment; a medical condition that required immunosuppression; or who had received more than 30 Gy of radiation in the thoracic region within the prior 26 weeks of initiation of study were ineligible. Randomization was stratified by ECOG performance status (0 vs. 1), histology (squamous vs. nonsquamous), geographic region (East Asia vs. non-East Asia), and PD-L1 expression (TPS \geq 50% vs. TPS 1 to 49%). Patients were randomized (1:1) to receive KEYTRUDA 200 mg intravenously every 3 weeks or investigator's choice of either of the following platinum-containing chemotherapy regimens:

- Pemetrexed 500 mg/m² every 3 weeks and carboplatin AUC 5 to 6 mg/mL/min every 3 weeks on Day 1 for a maximum of 6 cycles followed by optional pemetrexed 500 mg/m² every 3 weeks for patients with nonsquamous histologies;
- Paclitaxel 200 mg/m² every 3 weeks and carboplatin AUC 5 to 6 mg/mL/min every 3 weeks on Day 1 for a maximum of 6 cycles followed by optional pemetrexed 500 mg/m² every 3 weeks for patients with nonsquamous histologies.

Treatment with KEYTRUDA continued until RECIST v1.1 (modified to follow a maximum of 10 target lesions and a maximum of 5 target lesions per organ)-defined progression of disease, unacceptable toxicity, or a maximum of 24 months. Administration of KEYTRUDA was permitted beyond RECIST-defined disease progression if the patient was clinically stable and deriving clinical benefit as determined by the investigator. Treatment with KEYTRUDA could be reinitiated at the time of subsequent disease progression and administered for up to 12 months. Assessment of tumor status was performed every 9 weeks. The main efficacy outcome measure was OS. Additional efficacy outcome measures were PFS and ORR as assessed by a BICR review according to RECIST v1.1, modified to follow a maximum of 10 target lesions and a maximum of 5 target lesions per organ.

The study population characteristics were: median age of 63 years (range: 25 to 90), 45% age 65 or older; 71% male; 64% White, 30% Asian, and 2% Black. Nineteen percent were Hispanic or Latino. Sixty-nine percent had ECOG performance status of 1; 39% with squamous and 61% with nonsquamous histology; 87% with M1 disease and 13% with Stage IIIA (2%) or Stage IIIB (11%) who were not candidates for surgical resection or definitive chemoradiation per investigator assessment; and 5% with treated brain metastases at baseline. Forty-seven percent of patients had TPS \geq 50% NSCLC and 53% had TPS 1 to 49% NSCLC.

The trial demonstrated a statistically significant improvement in OS for patients randomized to KEYTRUDA as compared with chemotherapy. Table 5 and Figure 79 summarize the efficacy results in the subgroup of patients with TPS ≥ 50% and in all randomized patients with TPS ≥ 1%.

Table 5: Efficacy Results of All Randomized Patients (TPS ≥ 1% and TPS ≥ 50%) in KEYNOTE-042

Endpoint	TPS ≥ 1%		TPS ≥ 50%	
	KEYTRUDA 200 mg every 3 weeks n=637	Chemotherapy n=637	KEYTRUDA 200 mg every 3 weeks n=299	Chemotherapy n=300
OS				
Number of events (%)	371 (58%)	438 (69%)	157 (53%)	199 (66%)
Median in months (95% CI)	16.7 (13.9, 19.7)	12.1 (11.3, 13.3)	20.0 (15.4, 24.9)	12.2 (10.4, 14.2)
Hazard ratio* (95% CI)	0.81 (0.71, 0.93)		0.69 (0.56, 0.85)	
p-Value [†]	0.0036		0.0006	
PFS				
Number of events (%)	507 (80%)	506 (79%)	221 (74%)	233 (78%)
Median in months (95% CI)	5.4 (4.3, 6.2)	6.5 (6.3, 7.0)	7.1 (5.9, 9.0)	6.4 (6.1, 6.9)
Hazard ratio* [‡] (95% CI)	1.07 (0.94, 1.21)		0.81 (0.67, 0.99)	
p-Value [†]	- †		NS [§]	
Objective Response Rate				
ORR [‡] (95% CI)	27% (24, 31)	27% (23, 30)	39% (33.9, 45.3)	32% (26.8, 37.6)
Complete response rate	0.5%	0.5%	0.7%	0.3%
Partial response rate	27%	26%	39%	32%
Duration of Response				
% with duration ≥ 12 months [¶]	47%	16%	42%	17%
% with duration ≥ 18 months [¶]	26%	6%	25%	5%

* Based on the stratified Cox proportional hazard model

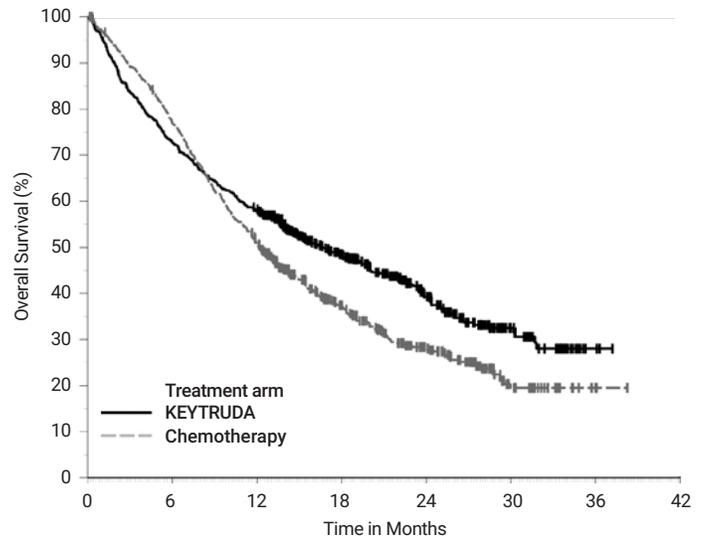
† Based on a stratified log-rank test; compared to a p-Value boundary of 0.0291

‡ Not evaluated for statistical significance as a result of the sequential testing procedure for the secondary endpoints

§ Not significant compared to a p-Value boundary of 0.0291

¶ Based on observed duration of response

In a pre-specified exploratory subgroup analysis for patients with TPS 1–49% NSCLC, the median OS was 13.4 months (95% CI: 10.7, 18.2) for the pembrolizumab group and 12.1 months (95% CI: 11.0, 14.0) in the chemotherapy group, with an HR of 0.92 (95% CI: 0.77, 1.11).



Number at Risk	0	6	12	18	24	30	36	42
KEYTRUDA:	637	463	365	214	112	35	2	0
Chemotherapy:	637	485	316	166	88	24	1	0

Figure 79: Kaplan-Meier Curve for Overall Survival in all Randomized Patients in KEYNOTE-042 (TPS ≥ 1%).

KEYNOTE-024: Controlled Trial of First-line Treatment of Patients with NSCLC

The efficacy of KEYTRUDA was investigated in Trial 24, a randomized (1:1), open-label, multicenter, controlled trial. Key eligibility criteria were metastatic NSCLC, PD-L1 expression tumor proportion score (TPS) of 50% or greater by an immunohistochemistry assay using PD-L1 IHC 22C3 pharmDx, and no prior systemic treatment for metastatic NSCLC. Patients with EGFR or ALK genomic tumor aberrations; autoimmune disease that required systemic therapy within 2 years of treatment; a medical condition that required immunosuppression; or who had received more than 30 Gy of thoracic radiation within the prior 26 weeks were ineligible. Patients were randomized to receive KEYTRUDA 200 mg every 3 weeks (n=154) or investigator's choice platinum-containing chemotherapy (n=151; including pemetrexed + carboplatin, pemetrexed + cisplatin, gemcitabine + cisplatin, gemcitabine + carboplatin, or paclitaxel + carboplatin. Non-squamous patients could receive pemetrexed maintenance). Patients were treated with KEYTRUDA until unacceptable toxicity or disease progression, or up to 35 administrations. Subsequent disease progression could be retreated for up to 1 additional year. Treatment could continue beyond disease progression if the patient was clinically stable and was considered to be deriving clinical benefit by the investigator. Assessment of tumor status was performed every 9 weeks. Patients on chemotherapy who experienced progression of disease were offered KEYTRUDA.

Among the 305 patients in Trial 24, baseline characteristics were: median age 65 years (54% age 65 or older); 61% male; 82% White and 15% Asian; and 35% and 65% with an ECOG performance status 0 and 1, respectively. Disease characteristics were squamous (18%) and non-squamous (82%); M1 (99%); and brain metastases (9%).

The major efficacy outcome measure was progression-free survival (PFS) as assessed by blinded independent central review (BICR) using Response Evaluation Criteria on Solid Tumors Version 1.1 (RECIST 1.1). Additional efficacy outcome measures were overall survival (OS) and objective response rate (ORR) as assessed by BICR using RECIST 1.1. Table 6 summarizes key efficacy measures for the entire intent to treat (ITT) population.

Table 6: Efficacy Results in Trial 24

Endpoint	KEYTRUDA 200 mg every 3 weeks n=154	Chemotherapy n=151
PFS*		
Number (%) of patients with event	73 (47%)	116 (77%)
Hazard ratio [†] (95% CI)	0.50 (0.37, 0.68)	
p-Value [‡]	<0.001	
Median in months (95% CI)	10.3 (6.7, NA)	6.0 (4.2, 6.2)
OS		
Number (%) of patients with event	44 (29%)	64 (42%)
Hazard ratio [†] (95% CI)	0.60 (0.41, 0.89)	
p-Value [‡]	0.005	
Median in months (95% CI)	Not reached (NA, NA)	Not reached (9.4, NA)
Objective Response Rate*		
ORR% (95% CI)	45% (37, 53)	28% (21, 36)
Complete response %	4%	1%
Partial response %	41%	27%

* Assessed by BICR using RECIST 1.1

[†] Hazard ratio (KEYTRUDA compared to chemotherapy) based on the stratified Cox proportional hazard model

[‡] Based on stratified Log rank test

NA = not available

Among the 69 patients randomized to KEYTRUDA 200 mg with an objective response, response durations ranged from 1.9+ to 14.5+ months. Eighty-eight percent of these responders had a response duration of 6 months or longer (based on Kaplan-Meier estimation; Figure 80).

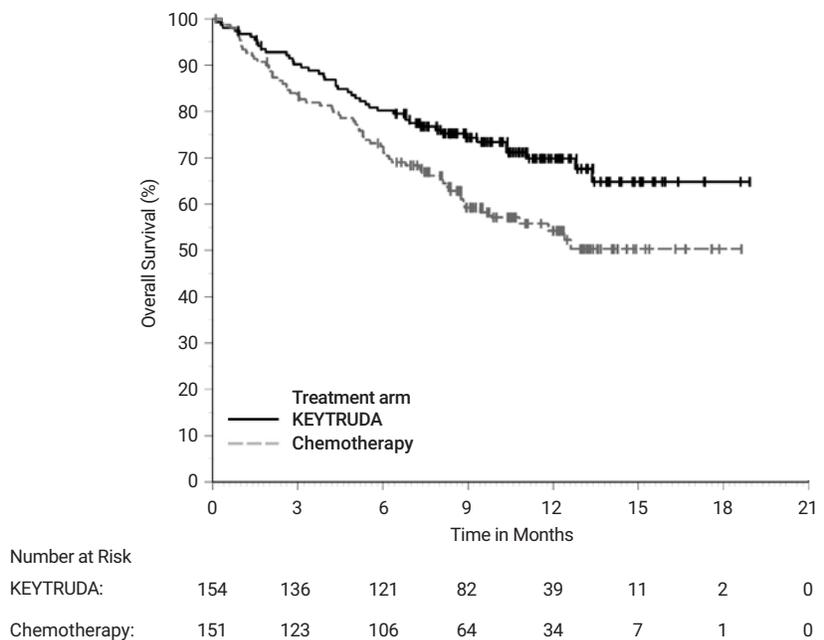


Figure 80: Kaplan-Meier Curve for Overall Survival in Trial 24.

KEYNOTE-010: Controlled Trial of NSCLC Patients Previously Treated with Chemotherapy

The efficacy of KEYTRUDA was investigated in Trial 10, a randomized (1:1), open-label, multicenter, controlled trial. Key eligibility criteria were advanced NSCLC that had progressed following platinum-containing chemotherapy, and if appropriate, targeted therapy for ALK or EGFR mutations, and PD-L1 expression tumor proportion score (TPS) of 1% or greater by a Clinical Trial Assay (CTA) version of PD-L1 IHC 22C3 pharmDx. Forty-four and 56 percent of patients were enrolled based on testing of an archival tumor sample or a new tumor sample, respectively. Patients with autoimmune disease; a medical condition that required immunosuppression; or who had received more than 30 Gy of thoracic radiation within the prior 26 weeks were ineligible. Patients were randomized (1:1:1) to receive 2 mg/kg (n=344) or 10 mg/kg (n=346) of KEYTRUDA every 3 weeks or 75 mg/m² of docetaxel every 3 weeks (n=343). Patients were treated with KEYTRUDA until unacceptable toxicity or disease progression that was symptomatic, was rapidly progressive, required urgent intervention, occurred with a decline in performance status, or was confirmed at 4 to 6 weeks with repeat imaging. Patients without disease progression were treated for up to 24 months or 35 administrations, whichever was longer. Subsequent disease progression could be retreated for up to 1 additional year. Assessment of tumor status was performed every 9 weeks. The primary efficacy outcome measures were OS and PFS as assessed by BICR using RECIST 1.1.

Based on the CTA, a total of 1,033 NSCLC patients were randomized in the study. To evaluate the clinical utility of PD-L1 IHC 22C3 pharmDx, archived clinical study samples were retrospectively tested at a USA-based reference laboratory with PD-L1 IHC 22C3 pharmDx. Out of the 1,033 patients, tumor tissue from 529 patients was retrospectively tested with PD-L1 IHC 22C3 pharmDx. Specimens from 413 patients had PD-L1 expression (≥ 1% of viable tumor cells exhibiting membrane staining at any intensity) and samples from 94 patients did not have PD-L1 expression (< 1% of viable tumor cells exhibiting membrane staining at any intensity). Within these 413 patients with PD-L1 expression, specimens from 163 patients had high PD-L1 expression (≥ 50% of viable tumor cells exhibiting membrane staining at any intensity).

The level of agreement achieved between the CTA and PD-L1 IHC 22C3 pharmDx is shown in Table 7.

Table 7: CTA vs. PD-L1 IHC 22C3 pharmDx Agreement

Agreement Rates	PD-L1 Cut-off	Negative Percent Agreement (95% Confidence Interval (CI))	Positive Percent Agreement (95% Confidence Interval (CI))
CTA vs. PD-L1 IHC 22C3 pharmDx	TPS ≥ 1%	94.5% [91.4%–96.6%]	80.0% [76.9%–82.8%]
	TPS ≥ 50%	98.3% [97.1%–99.0%]	73.2% [67.9%–77.9%]

Among randomized patients having PD-L1 expression by PD-L1 IHC 22C3 pharmDx, the demographic and other baseline characteristics were well balanced between the treatment arms. The median age was 63 years (44% age 65 or older). The majority of patients were white (77%) and male (58%); baseline ECOG

performance status was 0 (29%) or 1 (71%). Seventy-eight percent (78%) of patients were former/current smokers. Twenty-two percent (22%) of patients had squamous histology and 69% had non-squamous histology. The baseline and demographic characteristics were similarly well balanced across pembrolizumab and docetaxel arms in the overall clinical study.

Efficacy results are summarized in Tables 8 and 9. KEYTRUDA demonstrated durable clinical benefit in NSCLC patients with PD-L1 expression (TPS ≥ 1%), which was enhanced in patients with high PD-L1 expression (TPS ≥ 50%), as determined by PD-L1 IHC 22C3 pharmDx. The magnitude of benefit was comparable to that in the overall clinical trial. The tables below summarize key efficacy measures in the overall population with PD-L1 expression (TPS ≥ 1%) and in the high PD-L1 expression (TPS ≥ 50%) subset for the overall clinical study (TPS ≥ 1% by CTA) and in the population with PD-L1 expression by PD-L1 IHC 22C3 pharmDx. The Kaplan-Meier curve for OS (TPS ≥ 1%), as determined by PD-L1 IHC 22C3 pharmDx is shown in Figure 81. Efficacy results were similar for the 2 mg/kg and 10 mg/kg KEYTRUDA arms.

Table 8: Response to KEYTRUDA in Previously Treated NSCLC Patients: Overall Clinical Trial and Patients with PD-L1 Expression, TPS ≥ 1%, as determined by PD-L1 IHC 22C3 pharmDx

Endpoint	KEYTRUDA 2 mg/kg every 3 weeks		KEYTRUDA 10 mg/kg every 3 weeks		Docetaxel 75 mg/m ² every 3 weeks	
	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx
Number of patients	344	140	346	142	343	131
OS						
Deaths (%)	172 (50%)	59 (42%)	156 (45%)	59 (42%)	193 (56%)	67 (51%)
Hazard ratio* (95% CI)	0.71 (0.58, 0.88)	0.54 (0.37, 0.78)	0.61 (0.49, 0.75)	0.57 (0.39, 0.82)	-	-
p-Value [†]	<0.001	<0.001	<0.001	0.00115	-	-
Median in months (95% CI)	10.4 (9.4, 11.9)	11.8 (9.6, NA)	12.7 (10.0, 17.3)	12.0 (8.7, NA)	8.5 (7.5, 9.8)	7.5 (6.3, 9.9)
PFS[‡]						
Events (%)	266 (77%)	97 (63%)	255 (74%)	103 (73%)	257 (75%)	94 (72%)
Hazard ratio* (95% CI)	0.88 (0.73, 1.04)	0.68 (0.50, 0.92)	0.79 (0.66, 0.94)	0.79 (0.59, 1.06)	-	-
p-Value [†]	0.068	0.00578	0.005	0.05767	-	-
Median in months (95% CI)	3.9 (3.1, 4.1)	4.9 (4.1, 6.2)	4.0 (2.6, 4.3)	4.0 (2.2, 4.6)	4.0 (3.1, 4.2)	3.8 (2.2, 4.2)
Overall response rate[‡]						
ORR % [§] (95% CI)	18% (14, 23)	24% (17, 32)	18% (15, 23)	20% (14, 28)	9% (7, 13)	5% (2, 11)

* Hazard ratio (KEYTRUDA compared to docetaxel) based on the stratified Cox proportional hazard model

[†] Based on stratified Log rank test

[‡] Assessed by BICR using RECIST 1.1

[§] All responses were partial responses

Table 9: Response to KEYTRUDA in Previously Treated NSCLC Patients: Overall Clinical Trial and Patients with PD-L1 High Expression, TPS ≥ 50%, as determined by PD-L1 IHC 22C3 pharmDx

Endpoint	KEYTRUDA 2 mg/kg every 3 weeks		KEYTRUDA 10 mg/kg every 3 weeks		Docetaxel 75 mg/m ² every 3 weeks	
	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx
Number of patients	139	56	151	60	152	47
OS						
Deaths (%)	58 (42%)	18 (32%)	60 (40%)	19 (32%)	86 (57%)	25 (53%)
Hazard ratio* (95% CI)	0.54 (0.38, 0.77)	0.45 (0.24, 0.84)	0.50 (0.36, 0.70)	0.29 (0.15, 0.56)	-	-
p-Value [†]	<0.001	0.00541	<0.001	<0.001	-	-
Median in months (95% CI)	14.9 (10.4, NA)	Not reached (9.3, NA)	17.3 (11.8, NA)	Not reached (8.3, NA)	8.2 (6.4, 10.7)	7.2 (4.4, 8.3)
PFS[‡]						
Events (%)	89 (64%)	33 (59%)	97 (64%)	34 (57%)	118 (78%)	33 (70%)
Hazard ratio* (95% CI)	0.58 (0.43, 0.77)	0.47 (0.28, 0.80)	0.59 (0.45, 0.78)	0.41 (0.24, 0.70)	-	-
p-Value [†]	<0.001	0.00221	<0.001	<0.001	-	-
Median in months (95% CI)	5.2 (4.0, 6.5)	5.9 (4.2, 9.0)	5.2 (4.1, 8.1)	4.8 (2.8, NA)	4.1 (3.6, 4.3)	3.9 (2.0, 4.3)
Overall response rate[‡]						
ORR % [§] (95% CI)	30% (23, 39)	37% (25, 52)	29% (22, 37)	28% (18, 41)	8% (4, 13)	4% (1, 15)

* Hazard ratio (KEYTRUDA compared to docetaxel) based on the stratified Cox proportional hazard model

[†] Based on stratified Log rank test

[‡] Assessed by BICR using RECIST 1.1

[§] All responses were partial responses

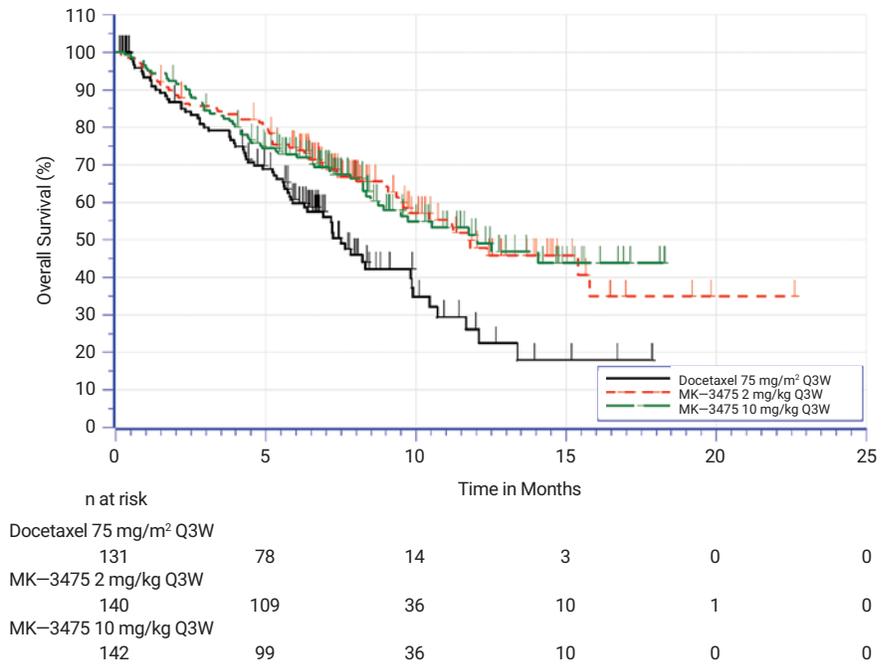


Figure 81: Kaplan-Meier Curve for Overall Survival by Treatment Arm (TPS ≥ 1% by PD-L1 IHC 22C3 pharmDx, Intent to Treat Population).

Additional robustness analyses were conducted to consider the potential impact of missing data arising from patients with PD-L1 expression (TPS ≥ 1%) by PD-L1 IHC 22C3 pharmDx, but who may have had no PD-L1 expression (TPS < 1%) by the CTA. Patients with such test results are part of the intended use/intent to diagnose (ITD)/population of PD-L1 IHC 22C3 pharmDx; however, they were excluded from the clinical trial due to no PD-L1 expression upon CTA screening. To account for these missing data, a sensitivity analysis was conducted to understand the plausible range for the hazard ratio (HR) estimated based on PD-L1 IHC 22C3 pharmDx in the TPS ≥ 1% and TPS ≥ 50% subpopulations under an ITD framework to verify the consistency with the observed HR based on enrollment with the CTA. The HR sensitivity analysis results showed that the HR estimates are robust to any assumed attenuation of the treatment effect under the ITD framework.

LIBTAYO® (cemiplimab-rwlc)**Regeneron EMPOWER-Lung 1: First-line Treatment of Locally Advanced NSCLC In Patients Who Are Not Candidates for Surgical Resection or Definitive Chemoradiation, or Metastatic NSCLC**

The efficacy of LIBTAYO in patients with locally advanced NSCLC who were not candidates for surgical resection or definitive chemoradiation, or with metastatic NSCLC was evaluated in a randomized, open-label, multi-center trial: EMPOWER-Lung 1 (NCT03088540).

The trial was designed to enroll patients with tumor PD-L1 expression of TPS \geq 50%. A total of 710 patients (Intent-To-Treat [ITT] population) were enrolled, and an analysis was performed on a population (n=563) who had PD-L1 expression of TPS \geq 50% using PD-L1 IHC 22C3 pharmDx according to the approved labeling.

Patients with EGFR, ALK or ROS1 genomic tumor aberrations; a medical condition that required systemic immunosuppression; autoimmune disease that required systemic therapy within 2 years of treatment; or who had never smoked were ineligible. Patients with a history of brain metastases were eligible if they had been adequately treated and had neurologically returned to baseline for at least 2 weeks prior to randomization.

Randomization was stratified by histology (non-squamous vs squamous) and geographic region (Europe vs Asia vs Rest of world). Patients were randomized (1:1) to receive LIBTAYO 350 mg intravenously (IV) every 3 weeks for up to 108 weeks or a platinum-doublet chemotherapy regimen for 4 to 6 cycles followed by optional pemetrexed maintenance for patients with non-squamous histology who received a pemetrexed containing regimen.

Treatment with LIBTAYO continued until RECIST 1.1-defined progressive disease, unacceptable toxicity, or up to 108 weeks. Patients who experienced IRC-assessed RECIST 1.1-defined progressive disease on LIBTAYO therapy were permitted to continue treatment with LIBTAYO (up to an additional 108 weeks) with the addition of 4 cycles of histology-specific chemotherapy until further progression was observed. Of the 150 patients in the population with TPS \geq 50% randomized to receive chemotherapy who had IRC-assessed RECIST 1.1-defined disease progression, 107 (71.3%) patients crossed over to treatment with LIBTAYO. Assessment of tumor status was performed every 9 weeks. The major efficacy outcome measures were overall survival (OS) and progression-free survival (PFS). An additional efficacy outcome measure was overall response rate (ORR).

The study population characteristics of patients with PD-L1 expression of TPS \geq 50% are included in Table 10.

Table 10: Summary of Baseline Patient and Disease Characteristics in the Population with TPS \geq 50%

	LIBTAYO N=283	Chemotherapy N=280
Patient Characteristics		
Median Age, Years (min, max)	63 (31, 79)	64 (40, 84)
Age < 65 Years, n (%)	157 (55)	147 (53)
Age \geq 65 Years, n (%)	126 (45)	133 (48)
Gender: Male n (%)	248 (88)	231 (83)
Race: White n (%)	243 (86)	240 (86)
ECOG Performance Status n (%)		
0	77 (27)	75 (27)
1	206 (73)	205 (73)
History of brain metastasis (%)	12	12
Disease Characteristics		
Extent of Disease n (%)		
Locally Advanced	45 (16)	42 (15)
Metastatic	238 (84)	238 (85)
Histological Subtype n (%)		
Squamous	122 (43)	121 (43)
Non-squamous	161 (57)	159 (57)

In the ITT population, baseline patient and disease characteristics were consistent with those in the population with TPS \geq 50%.

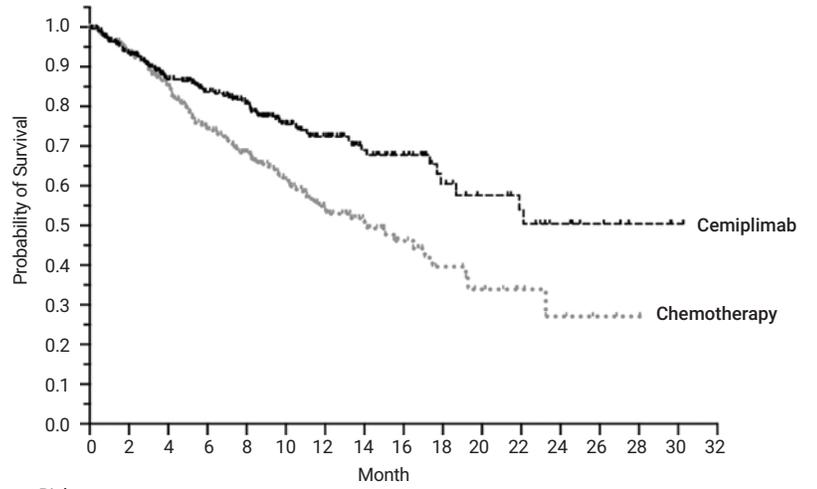
In the population with TPS \geq 50%, the trial demonstrated statistically significant improvement in OS and PFS for patients randomized to LIBTAYO as compared with chemotherapy. Results were similar to the efficacy results for the ITT population.

Efficacy results for the population with PD-L1 expression of TPS \geq 50% are presented in Table 11 and in Figure 82.

Table 11: Efficacy Results from EMPOWER-Lung 1 in Non-small Cell Lung Cancer

Endpoints	TPS ≥ 50% Population (N=563)	
	LIBTAYO 350 mg every 3 weeks n=283	Chemotherapy n=280
Overall Survival		
Number of deaths (%)	70 (24.7)	105 (37.5)
Median in months (95% CI) ^a	NR (17.9, NE)	14.2 (11.2, 17.5)
Hazard ratio (95% CI) ^b	0.57 (0.42, 0.77)	
p-Value	0.0002	
Progression-free Survival per BICR		
Number of events (%)	147 (51.9)	197 (70.4)
Median in months (95% CI) ^a	8.2 (6.1, 8.8)	5.7 (4.5, 6.2)
Hazard ratio (95% CI) ^b	0.54 (0.43, 0.68)	
p-Value	<0.0001	
Overall Response Rate per BICR (%)^{c,d}		
ORR (95% CI)	39.2 (33.5, 45.2)	20.4 (15.8, 25.6)
Complete response (CR) rate	2.1	1.1
Partial response (PR) rate	37.1	19.3
Duration of Response per BICR^e		
Median in months (range)	16.7 (1.9+, 23.3+)	6.0 (1.3+, 14.5+)

BICR: blinded independent central review; CI: Confidence interval; NE: Not evaluable; NR: Not reached
 +: Ongoing response
^a Based on Kaplan-Meier method
^b Based on stratified proportional hazards model
^c Not a pre-specified endpoint
^d Clopper-Pearson exact confidence interval



Number of Subjects at Risk

Cemiplimab:	283	244	203	177	154	108	83	55	42	24	18	15	10	6	3	1	0
Chemotherapy:	280	239	198	153	125	87	57	41	25	15	11	6	4	2	1	0	0

Figure 82: Kaplan-Meier Curve for OS in the TPS \geq 50% Population.

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For NSCLC,
Choose PD-L1 IHC 22C3 pharmDx
to identify patients for treatment
with KEYTRUDA or LIBTAYO



About This Manual

This Interpretation Manual provides a guide for high-quality staining and diagnostic assessment using PD-L1 IHC 22C3 pharmDx, through:

- Detailed scoring guidelines and technical instructions
- Example cases and in-depth recommendations for interpretation

About PD-L1 IHC 22C3 pharmDx

PD-L1 IHC 22C3 pharmDx is a qualitative IHC assay and is the only PD-L1 indicated as an aid in identifying patients with NSCLC for treatment with KEYTRUDA or LIBTAYO



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For countries outside of the United States, see the local KEYTRUDA product label for approved indications and expression cutoff values to guide therapy.

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