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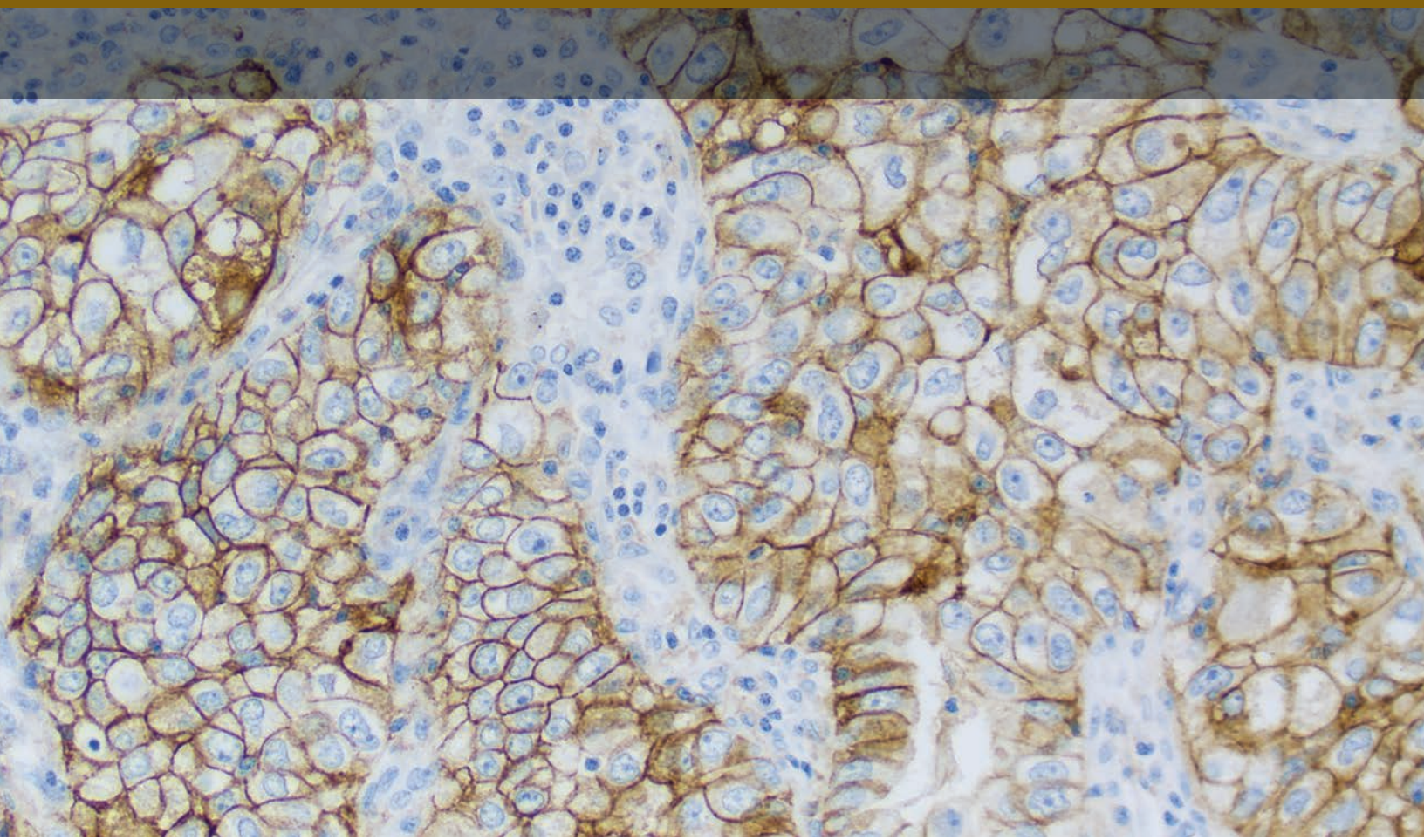
PD-L1 IHC 22C3 pharmDx Interpretation Manual – Non-small Cell Lung Cancer (NSCLC)

CE-IVD–marked for in vitro diagnostic use



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Trusted Answers

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

For countries outside of the European Union, see the local LIBTAYO product label for approved indications.

Table of Contents

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), urothelial carcinoma, head and neck squamous cell carcinoma (HNSCC), and melanoma 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 protein expression in urothelial carcinoma is determined by using Combined Positive Score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100.

PD-L1 protein expression in HNSCC is determined by using CPS and/or TPS.

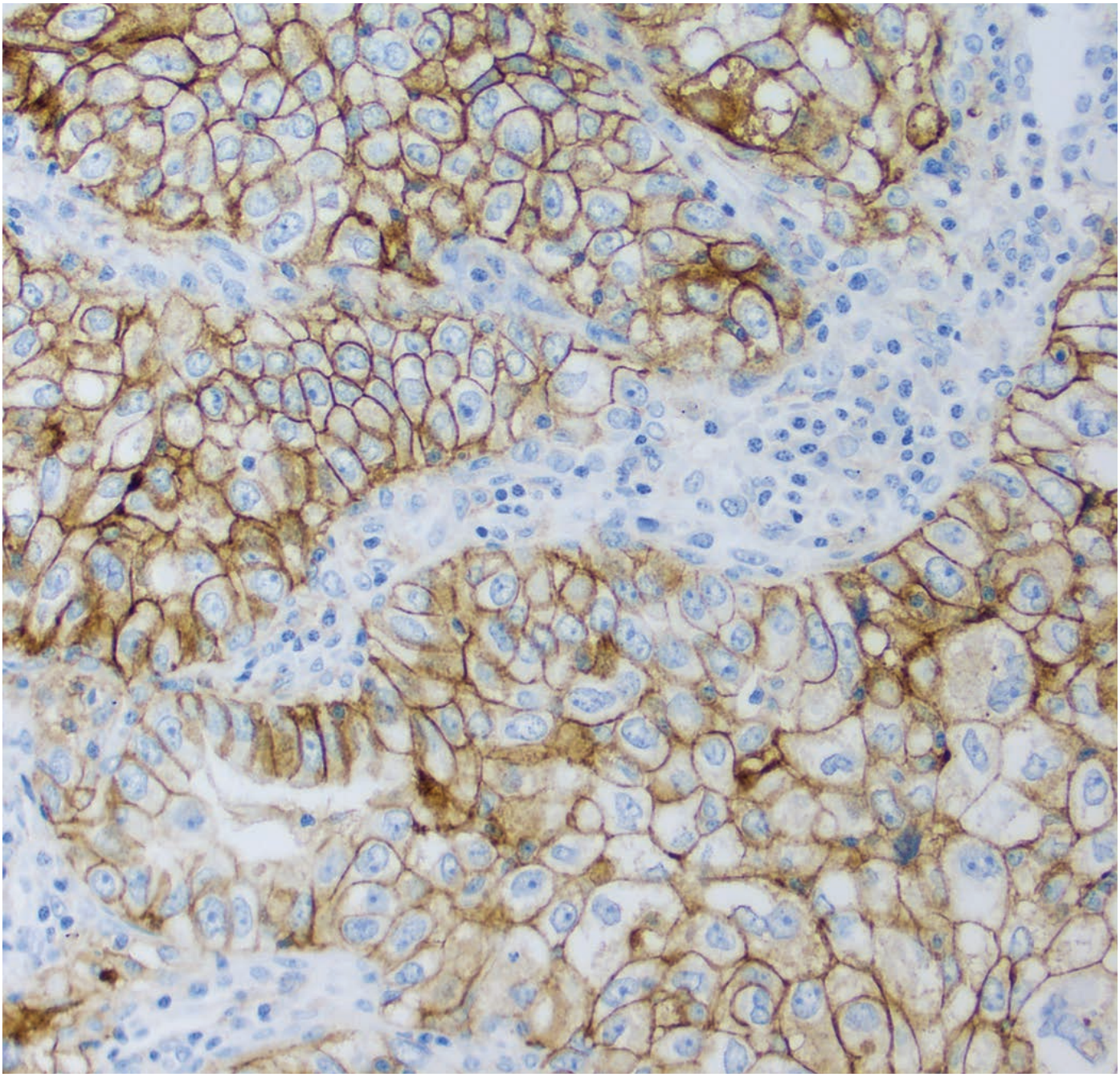
PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying patients for treatment with the therapies for the indications listed in Table 1.

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

Tumor Indication	PD-L1 Expression Level	Therapy
NSCLC	TPS ≥ 1% TPS ≥ 50%	KEYTRUDA®*
Urothelial Carcinoma	CPS ≥ 10	
HNSCC	CPS ≥ 1 TPS ≥ 50%	
NSCLC	TPS ≥ 50%	LIBTAYO®**

** See the KEYTRUDA® product label for PD-L1 expression cutoff values and specific clinical circumstances guiding therapy.*

*** See the LIBTAYO® product label for specific clinical circumstances guiding therapy.*



Introduction

PD-L1 IHC 22C3 pharmDx is the companion diagnostic CE-IVD–marked as an aid in identifying patients with NSCLC for treatment with KEYTRUDA® (pembrolizumab) or LIBTAYO® (cemiplimab) that was proven in their respective clinical trials. 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 specimens stained 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%
- TPS ≥ 50%

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

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.

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)

PD-L1 IHC 22C3 pharmDx was the companion diagnostic used in the LIBTAYO clinical trial (Regeneron Study 1624) 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

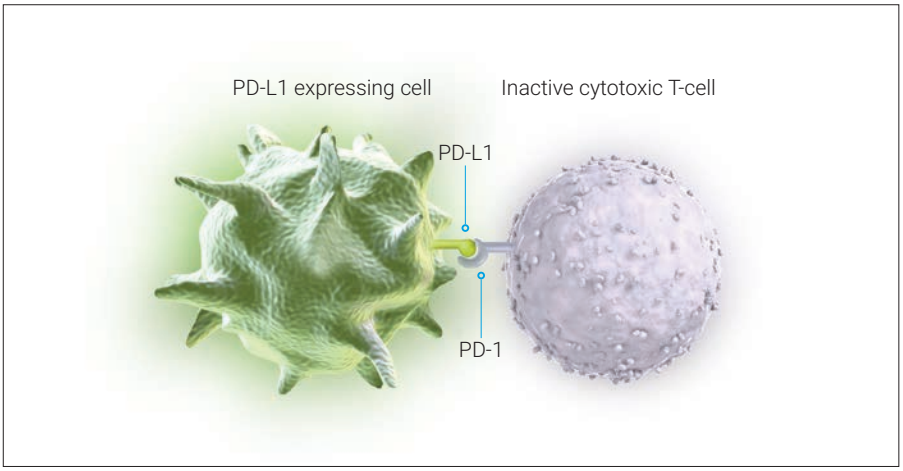


Figure 1: Inactivation of T-cells limits damage to normal tissue.

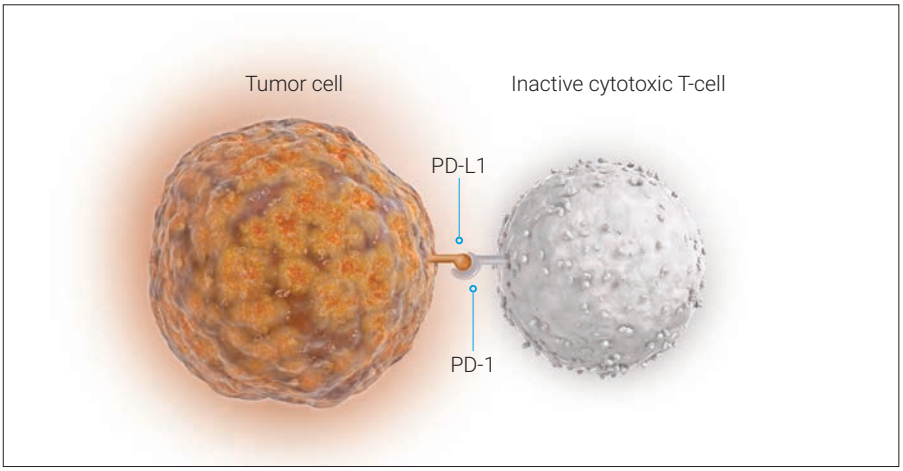


Figure 2: Inactivation of T-cells reduces tumor cell death and elimination.

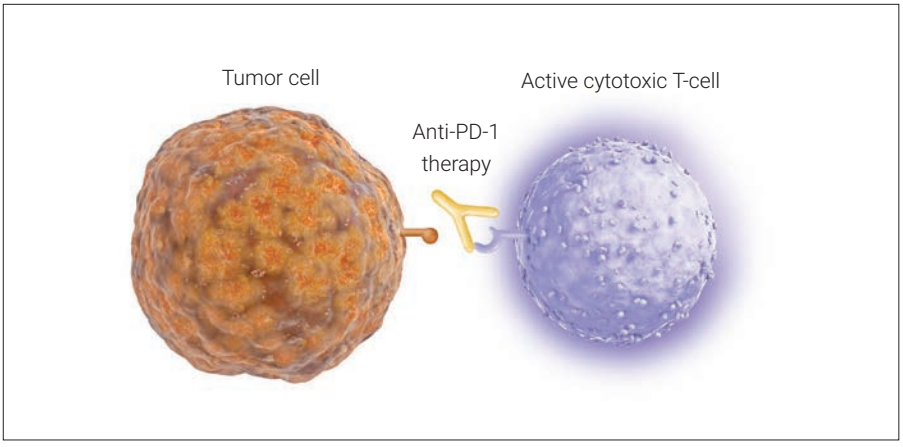


Figure 3: Blocking the PD-1/PD-L1 interaction helps to enable active T-cells and tumor cell death and elimination.

PD-L1 IHC 22C3 pharmDx Overview

What is PD-L1 IHC 22C3 pharmDx?

PD-L1 IHC 22C3 pharmDx is the companion diagnostic CE-IVD–marked as an aid in identifying patients with NSCLC for treatment with KEYTRUDA® (pembrolizumab) or LIBTAYO® (cemiplimab) that was proven in their respective clinical trials. 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.

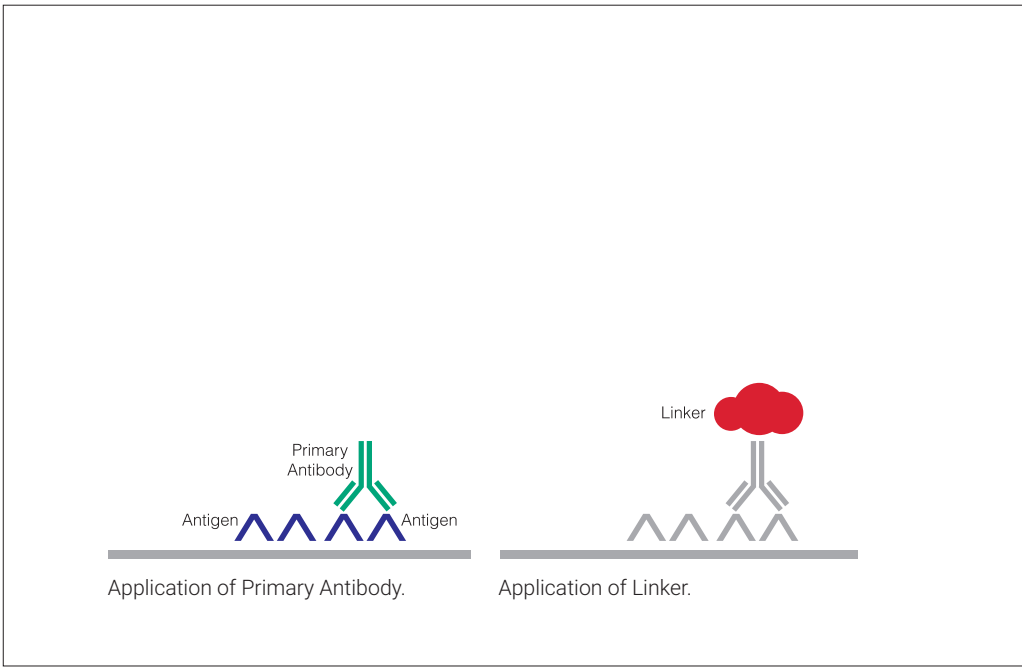


Figure 4: PD-L1 IHC 22C3 pharmDx staining procedure.

Kit Configuration (SK006)

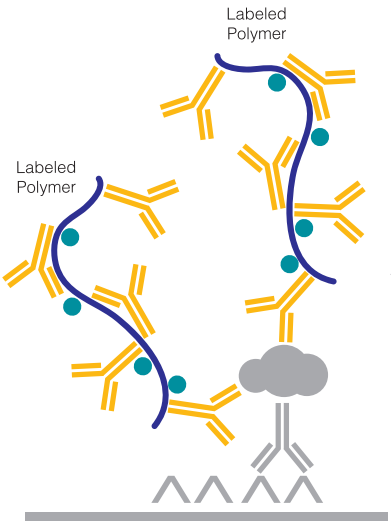
Figure 5: PD-L1 IHC 22C3 pharmDx components.

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

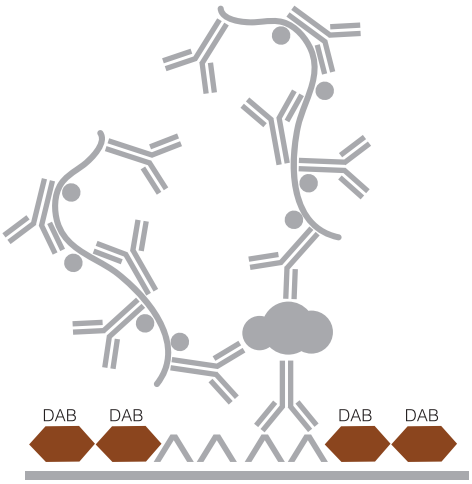
1. EnVision FLEX Target Retrieval Solution, Low pH (50×)
2. Peroxidase-blocking Reagent
3. Primary Antibody: Monoclonal Mouse Anti-PD-L1, Clone 22C3
4. Negative Control Reagent
5. Mouse LINKER
6. Visualization Reagent-HRP
7. DAB+ Substrate Buffer
8. DAB+ Chromogen
9. DAB Enhancer
10. PD-L1 IHC 22C3 pharmDx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

EnVision FLEX Wash Buffer (20×) (Code K8007) and Hematoxylin (Link) (Code K8008) are required but not included in the kit.

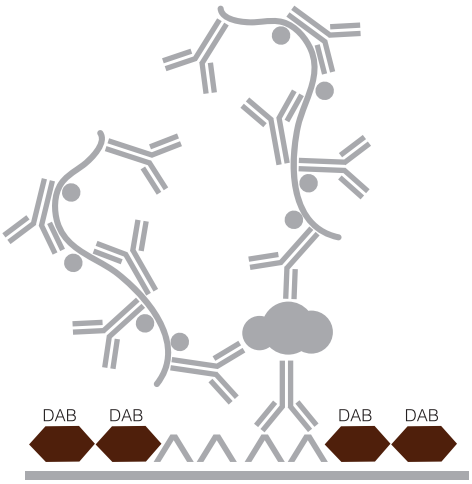
** Dr. AF Gazdar and Dr. JD Minna at NIH are acknowledged for their contribution in developing NCI-H226 (ATCC Number: CRL-5826™)*



Application of Visualization Reagent.



Application of DAB+ Substrate-Chromogen Solution.



Application of DAB Enhancer.

Technical Considerations

Technical problems related to PD-L1 IHC 22C3 pharmDx may arise and can be attributed to two areas: 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.

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 in-house control tissues stained with the primary antibody and Negative Control Reagant
- Serial section of each patient specimen stained with the Negative Control Reagent

In-house Control Tissue

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

Select positive and negative control tissues from fresh specimens of the same tumor indication as the patient specimen. Fix, process, and embed the control tissues 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 In-house 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. Store tissue sections in the dark at 2–8 °C (preferred) or at room temperature up to 25 °C to preserve antigenicity, and stain within 6 months of sectioning.

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-grade 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-grade water). Store unused 1× EnVision FLEX Wash 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 Solution 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

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 1× EnVision FLEX Target Retrieval Solution, Low pH to 65 °C
- Immerse Autostainer racks containing mounted, FFPE tissue sections into the preheated 1× EnVision FLEX 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 rack with slides on the Autostainer Link 48
- Ensure slides remain wet with buffer while loading and prior to initiating the run. Dried tissue sections may display increased non-specific staining
- 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 Hematoxylin (Link) (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?	☐	☐
PD-L1 IHC 22C3 pharmDx is used before the expiration date printed on the outside of the box?	☐	☐
All PD-L1 IHC 22C3 pharmDx components, including Control Cell Line Slides, are stored in the dark at 2–8 °C?	☐	☐
All PD-L1 IHC 22C3 pharmDx components, including Control Cell Line Slides, are equilibrated to room temperature (20–25 °C) prior to immunostaining?	☐	☐
Appropriate positive and negative control tissue from NSCLC are identified?	☐	☐
Tissues are fixed in neutral buffered formalin?	☐	☐
Tissues are infiltrated with melted paraffin, at or below 60 °C?	☐	☐
Tissue sections of 4–5 µm are mounted on Dako FLEX IHC Microscope Slides or Superfrost Plus slides?	☐	☐
Specimens are oven-dried at 58 ± 2 °C for 1 hour?	☐	☐
Specimens are stained within 6 months of sectioning when stored in the dark at 2–8 °C (preferred) or at room temperature up to 25 °C?	☐	☐
1× EnVision FLEX Target Retrieval Solution, Low pH is prepared properly? pH of 1× EnVision FLEX Target Retrieval Solution must be 6.1 ± 0.2.	☐	☐
1× EnVision FLEX Wash Buffer is prepared properly?	☐	☐
DAB+ Substrate-Chromogen Solution is prepared properly?	☐	☐
Slides are counterstained with Hematoxylin (Link) (Code K8008)?	☐	☐
The Deparaffinization, Rehydration, and Target Retrieval 3-in-1 procedure is followed using PT Link?	☐	☐
Slides remain wet with buffer while loading and prior to initiating run on Autostainer Link 48?	☐	☐
The PD-L1 IHC 22C3 pharmDx protocol is selected on Autostainer Link 48?	☐	☐
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.	☐	☐

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. In-house 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

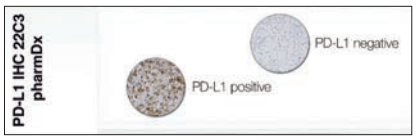


Figure 6: Each Control Cell Line Slide contains sections of cell pellets with positive and negative PD-L1 expression.

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 non-specific 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 ≥ 70% of cells
- ≥ 2+ average staining intensity
- Non-specific staining < 1+ intensity

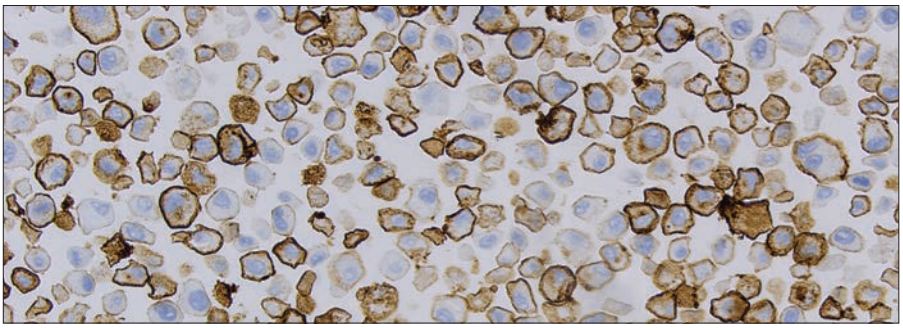


Figure 7: Positive cell pellet with acceptable staining of PD-L1 IHC 22C3 pharmDx Control Cell Line Slide (20× magnification).

Negative Control Cell Pellet

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

- No specific staining
- Non-specific 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 plasma membrane staining, or cytoplasmic staining with ≥ 1+ intensity within the boundaries of the MCF-7 cell pellet are acceptable

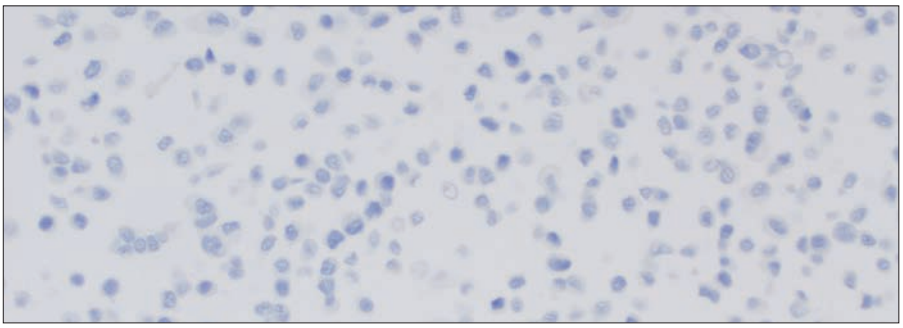


Figure 8: Negative cell pellet with no staining of PD-L1 IHC 22C3 pharmDx Control Cell Line Slide (20× magnification).

See the Control Cell Line (CCL) Appendix on page 70 for images of passing, borderline, and failing control cell line staining.

Positive and Negative In-house Control Tissue (NSCLC)

Examine the positive in-house NSCLC 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 in-house control tissue is not satisfactory, all results with the patient specimen should be considered invalid.

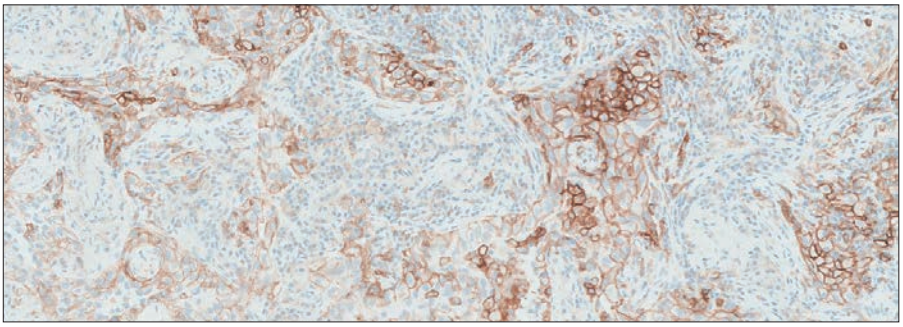


Figure 9: Positive in-house control tissue (10× magnification).

The ideal NSCLC 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 in-house 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 in-house control tissues, results with the patient specimen should be considered invalid.

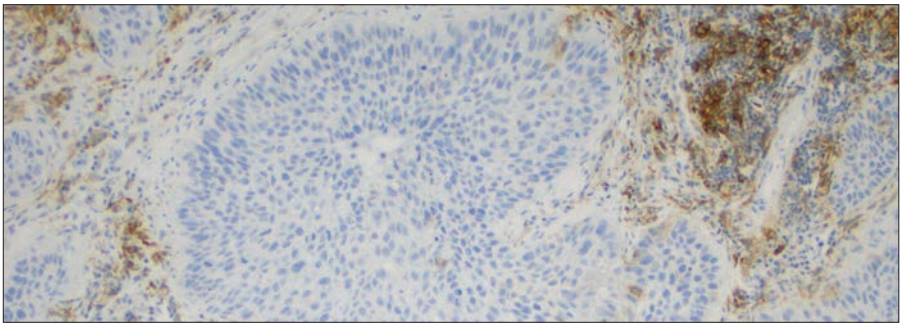


Figure 10: Negative in-house control tissue demonstrating lack of staining of tumor cells (10× magnification).

Optional Control Tissue

In addition to the Control Cell Line Slide and in-house 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.

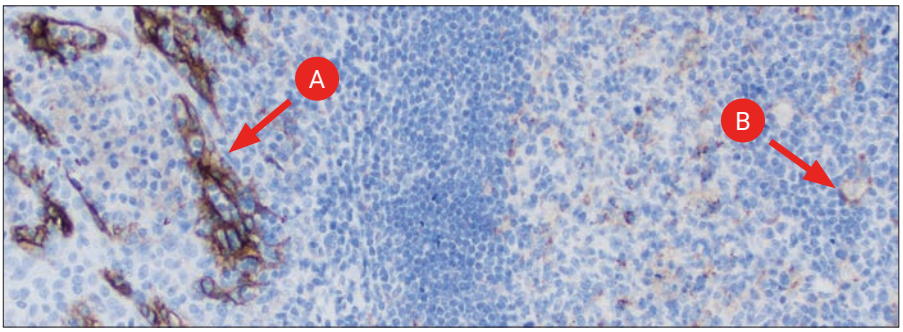


Figure 11: Tonsil stained with PD-L1 primary antibody exhibiting strong membrane staining in portions of the crypt epithelium (A) and weak-to-moderate membrane staining of follicular macrophages in the germinal centers (B) (10× magnification).

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

Negative Control Reagent (NCR)

Examine the slides stained with the NCR to identify non-specific background 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 non-specific staining that may interfere with interpreting the PD-L1 stained slide. Non-specific staining should be ≤ 1+.

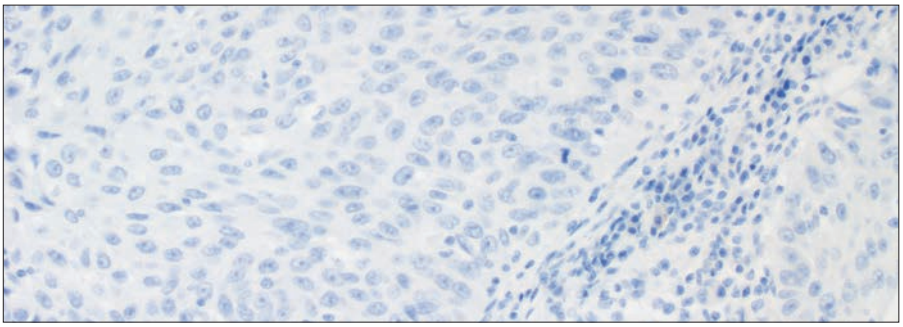


Figure 12: NSCLC tissue specimen stained with NCR (20× magnification).

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

Slide Evaluation Flowchart

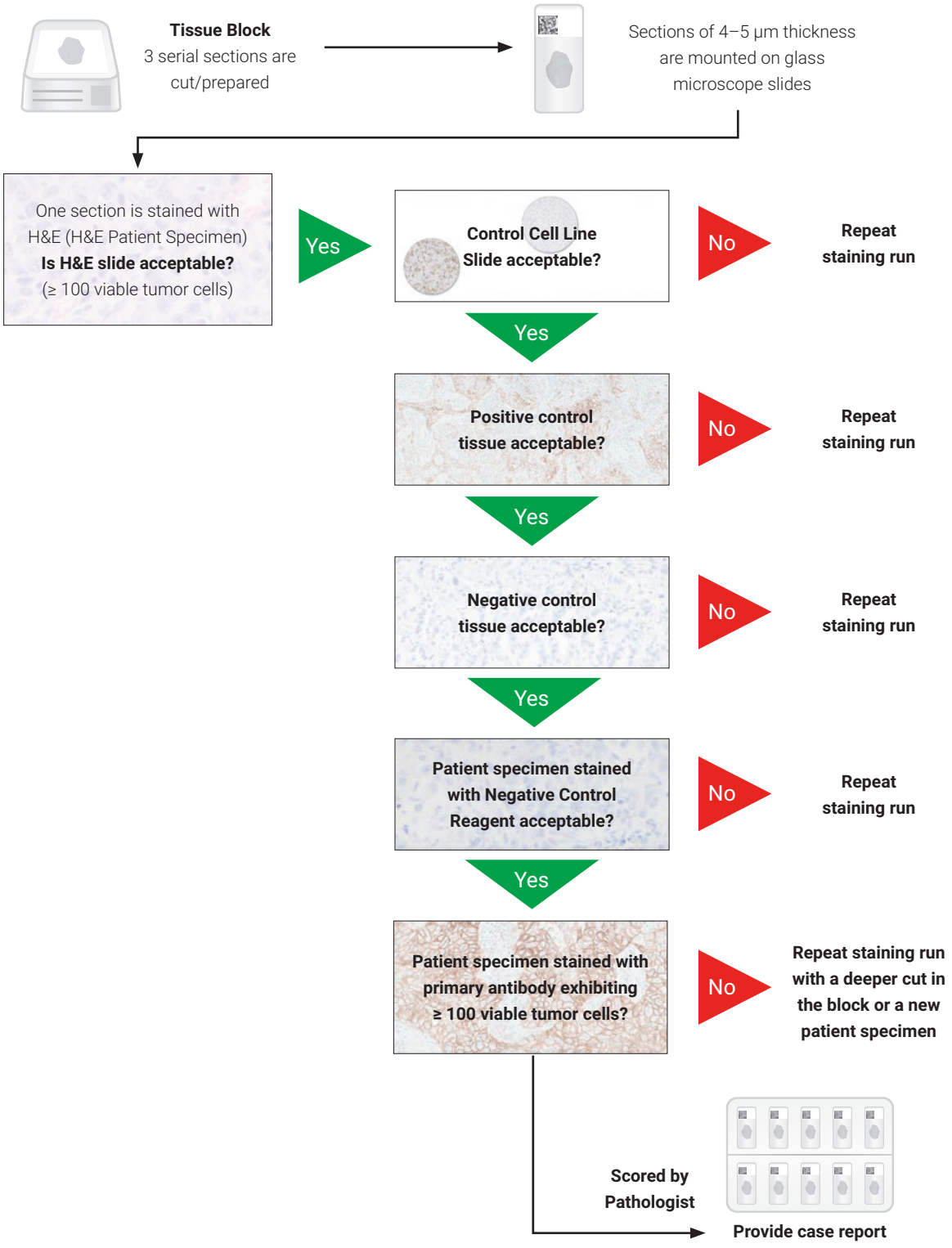


Figure 13: Recommended order of slide evaluation.

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 (≥ 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 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: – Mononuclear inflammatory cells (large lymphocytes, monocytes, pulmonary macrophages) – Plasma cells – Neutrophils
Other	Not included	Exclude any staining of: – 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 (≥ 1+) of tumor cells that is perceived distinct from cytoplasmic staining. Cytoplasmic staining should be considered non-specific staining and is excluded in the assessment of staining intensity.

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 ≥ 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×, 20×, and 40×, 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 non-staining tumor areas
  - Determining partial and complete membrane staining (≥ 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

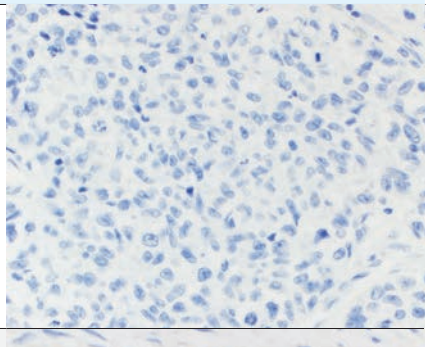
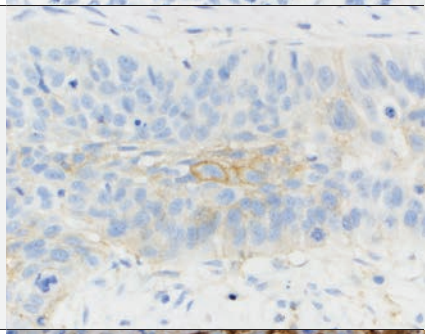
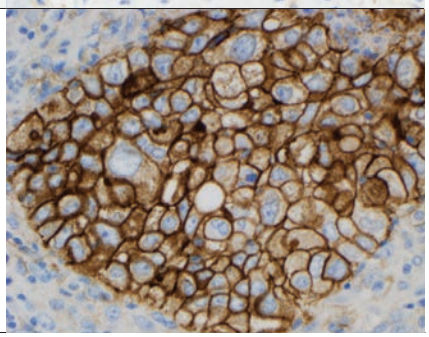
The following considerations can help distinguish tumor cells from immune cells:

- 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.

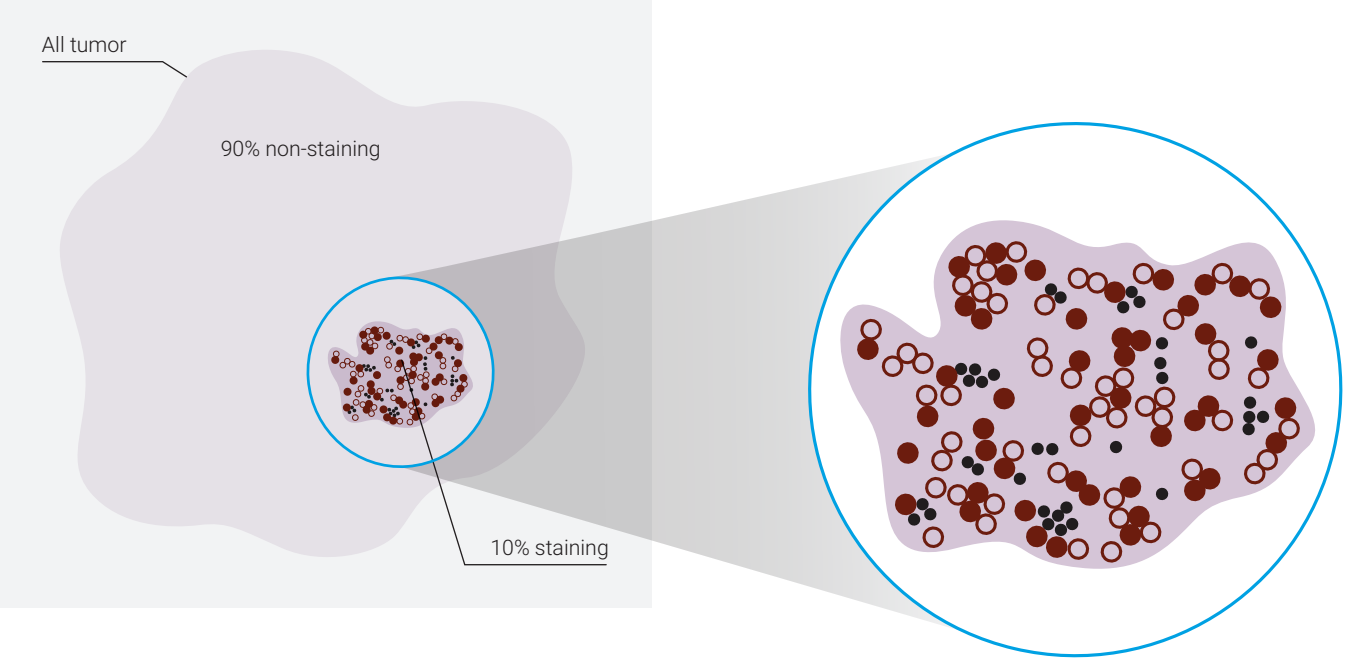
Example 1: Calculation of Tumor Proportion Score Based on a Small PD-L1 Staining Area

At lower magnification: Evaluate the tumor area for any perceptible and convincing ≥ 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% × 50% = 5%

Clinical Interpretation: TPS ≥ 1%

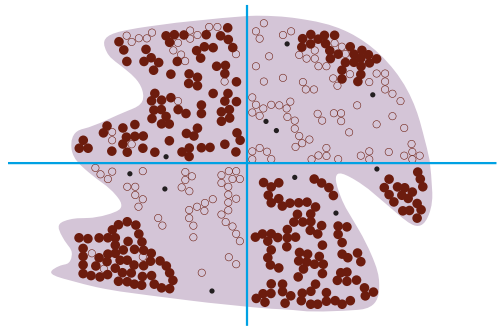
Figure 14: Example of tumor with small PD-L1 staining area.

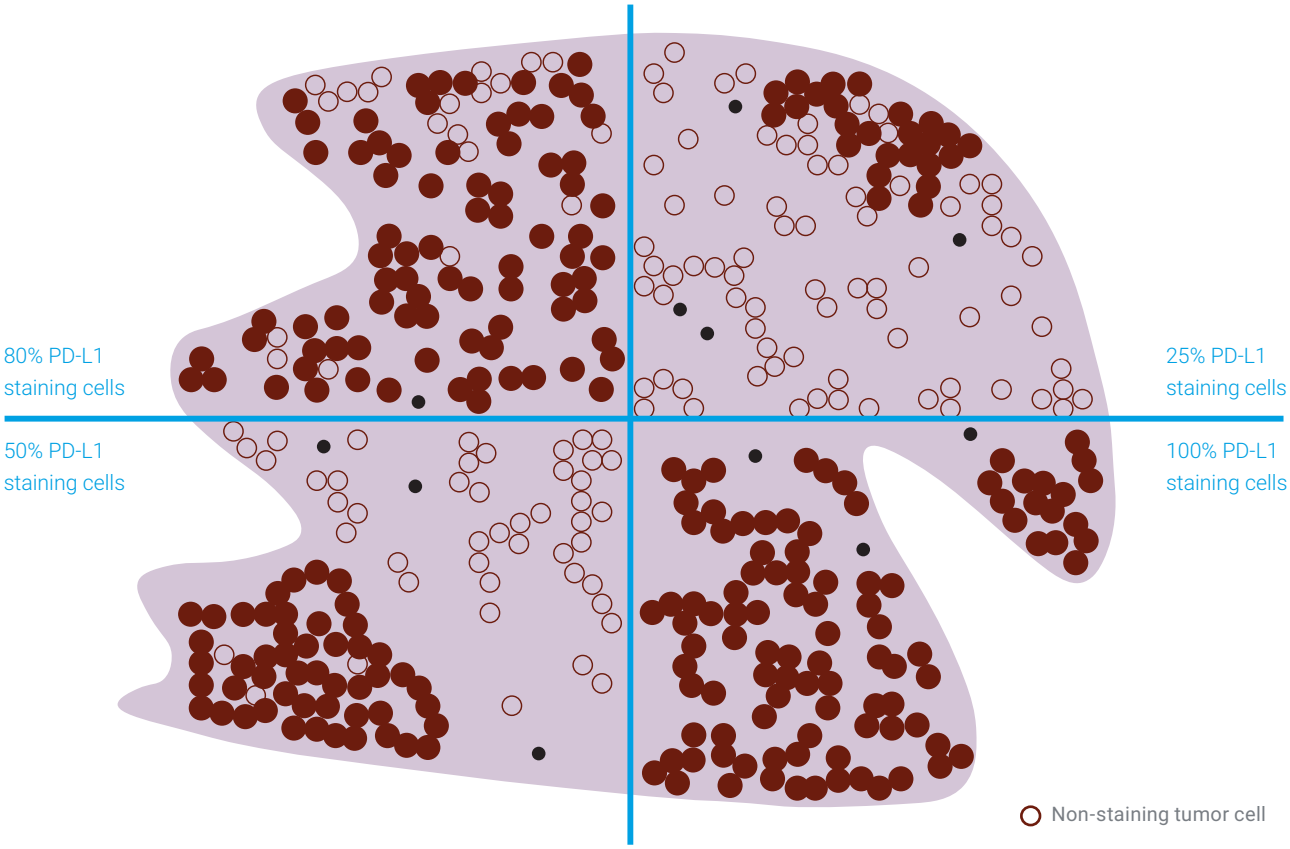
Example 2: Calculation of Tumor Proportion Score Based on a Heterogeneous PD-L1 Staining Area

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 ≈ 60%

Clinical Interpretation: TPS ≥ 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 first clinical trial-proven companion diagnostic indicated as an aid in identifying patients with NSCLC for treatment with KEYTRUDA monotherapy.

Clinical Validation of PD-L1 IHC 22C3 pharmDx in Previously Untreated Patients with Metastatic NSCLC (First-line)

The clinical validity of PD-L1 IHC 22C3 pharmDx in identifying PD-L1 expression (TPS ≥ 50%) in previously untreated patients with metastatic NSCLC is based on the KEYTRUDA KEYNOTE-024 study sponsored by Merck Sharp & Dohme Corp. Specimens from previously untreated patients with NSCLC were tested for PD-L1 expression using PD-L1 IHC 22C3 pharmDx. Only patients with TPS ≥ 50% were included in the KEYNOTE-024 study.

Table 4: PD-L1 Prevalence[a] in Patients with NSCLC[b] Screened for KEYNOTE-024[c]

PD-L1 Expression	TPS < 1%	TPS 1–49%	TPS ≥ 50%
Prevalence % (n)	30.7% (507)	39.1% (646)	30.2% (500)

a. Merck & Co., data on file
b. Patients screened for enrollment in KEYNOTE-024 NSCLC
c. International phase 3 study comparing pembrolizumab with investigator's choice platinum containing (including pemetrexed+carboplatin, pemetrexed+cisplatin, gemcitabine+cisplatin, gemcitabine+carboplatin, or paclitaxel+carboplatin) in patients with non-small cell lung carcinoma who were previously untreated for advanced metastatic disease. ClinicalTrials.gov number NCT02142738

Clinical Validation of PD-L1 IHC 22C3 pharmDx in Previously Treated Patients with Metastatic NSCLC (Second-line and Beyond)

The clinical validity of PD-L1 IHC 22C3 pharmDx in identifying PD-L1 expression (TPS ≥ 1%) in previously treated patients with NSCLC is based on the KEYTRUDA KEYNOTE-010 study sponsored by Merck Sharp & Dohme Corp. Specimens from previously treated patients with metastatic NSCLC were tested for PD-L1 expression using PD-L1 IHC 22C3 pharmDx. Only patients with TPS ≥ 1% were included in the KEYNOTE-010 study.

Table 5: PD-L1 Prevalence[d] in Patients with NSCLC[e] Screened for KEYNOTE-010[f]

PD-L1 Expression	TPS < 1%	TPS 1–49%	TPS ≥ 50%
Prevalence % (n)	43.0% (433)	34.2% (344)	22.8% (230)

d. Merck & Co., data on file
e. Patients screened for enrollment in KEYNOTE-010 NSCLC
f. International phase 2/3 study comparing pembrolizumab with docetaxel in patients with non-small cell lung carcinoma who have experienced disease progression after platinum-containing system therapy ClinicalTrials.gov number NCT01905657

Note: PD-L1 testing with PD-L1 IHC 22C3 pharmDx was used to qualify patients with NSCLC for first-line treatment with KEYTRUDA monotherapy in the KEYNOTE-042 clinical trial. For more information on the KEYNOTE clinical trials, review the Instructions for Use. Clinical efficacy of KEYTRUDA treatment is also presented in the Clinical Performance Evaluation section on pages 83–92.

PD-L1 IHC 22C3 pharmDx Testing Scheme

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

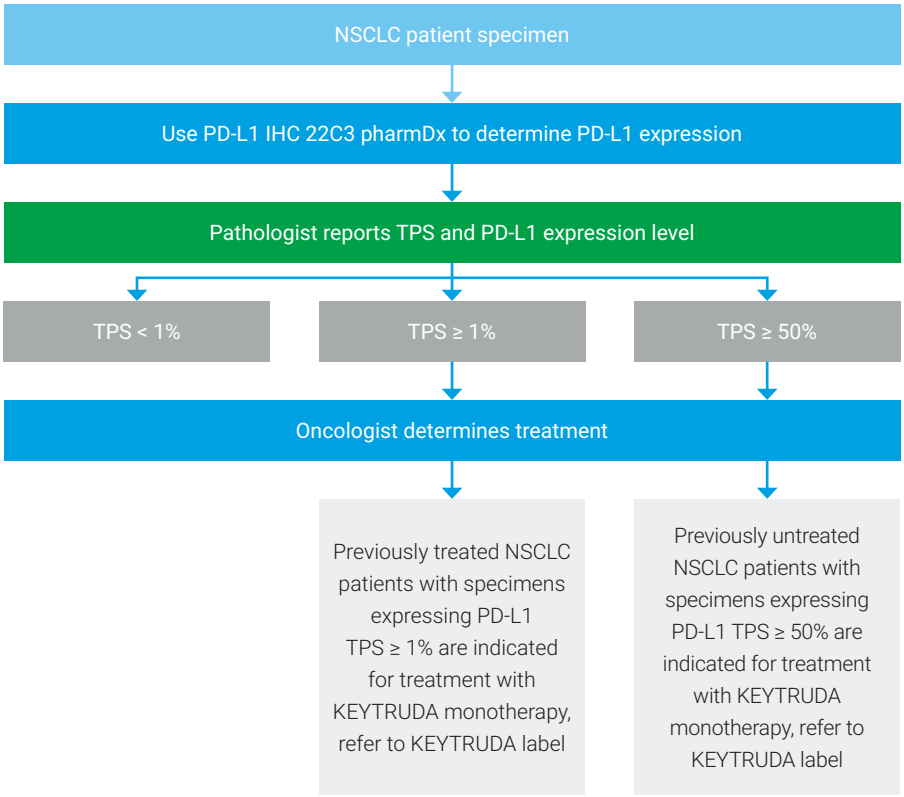


Figure 16: Testing scheme for PD-L1 IHC 22C3 pharmDx.

LIBTAYO® (cemiplimab)

PD-L1 IHC 22C3 pharmDx is the first 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 ≥ 50%) in patients with NSCLC is based on Study 1624, 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 ≥ 50% by PD-L1 IHC 22C3 pharmDx is presented in the Clinical Performance Evaluation section on pages 93–96.

PD-L1 IHC 22C3 pharmDx Testing Scheme

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

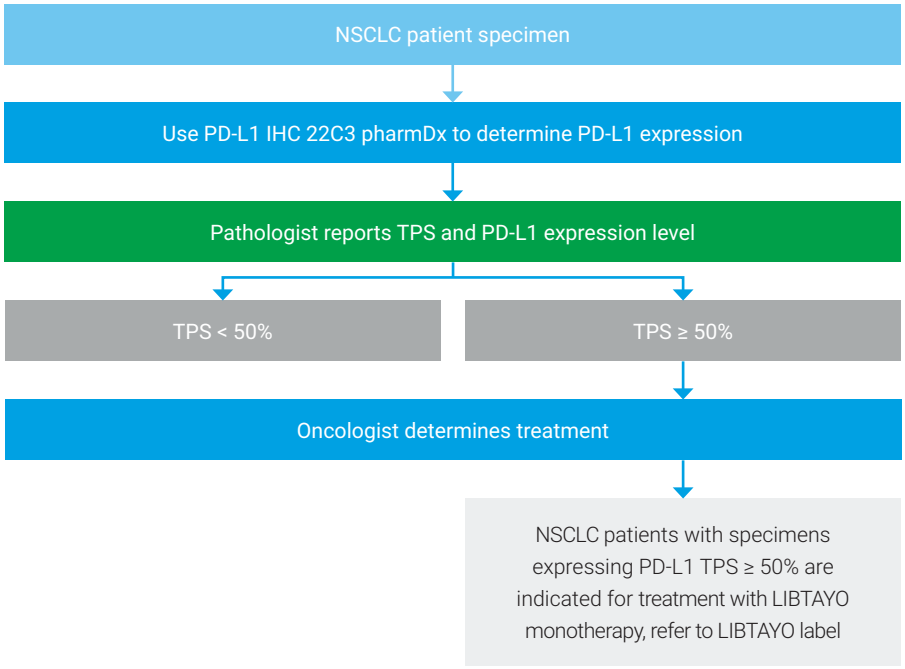


Figure 17: Testing scheme for PD-L1 IHC 22C3 pharmDx.

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 ≥ 1%: ☐ TPS ≥ 50%: ☐

Comments to Treating Physician:

- KEYTRUDA® (pembrolizumab) as monotherapy is indicated for the first-line treatment of metastatic non-small cell lung carcinoma (NSCLC) in adults whose tumors express PD-L1 with a ≥ 50% Tumor Proportion Score (TPS) with no EGFR or ALK positive tumor mutations
- KEYTRUDA as monotherapy is indicated for the treatment of locally advanced or metastatic NSCLC in adults whose tumors express PD-L1 with a ≥ 1% TPS and who have received at least one prior chemotherapy regimen. Patients with EGFR or ALK positive tumor mutations should also have received targeted therapy before receiving KEYTRUDA
- LIBTAYO® (cemiplimab) as monotherapy is indicated for the first-line treatment of adult patients with non-small cell lung cancer (NSCLC) expressing PD-L1 (in ≥ 50% tumor cells), with no EGFR, ALK or ROS1 aberrations, who have (1) locally advanced NSCLC who are not candidates for definitive chemoradiation, or (2) metastatic NSCLC

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 in the PD-L1 stained slide 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 10×, 20×, and 40× 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 (≥ 1+) and at any magnification. Review at higher magnification may be needed to confirm perceptible and convincing membrane staining.

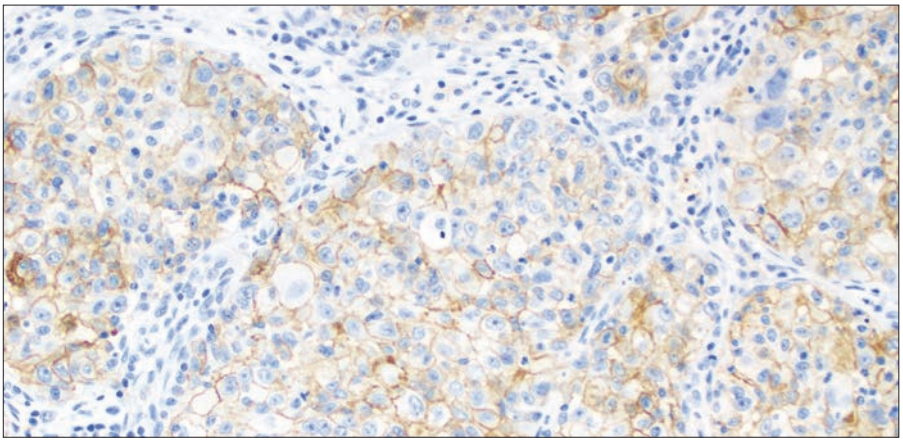


Figure 18a: NSCLC specimen stained with PD-L1 primary antibody exhibiting weak membrane staining of tumor cells (10× magnification).

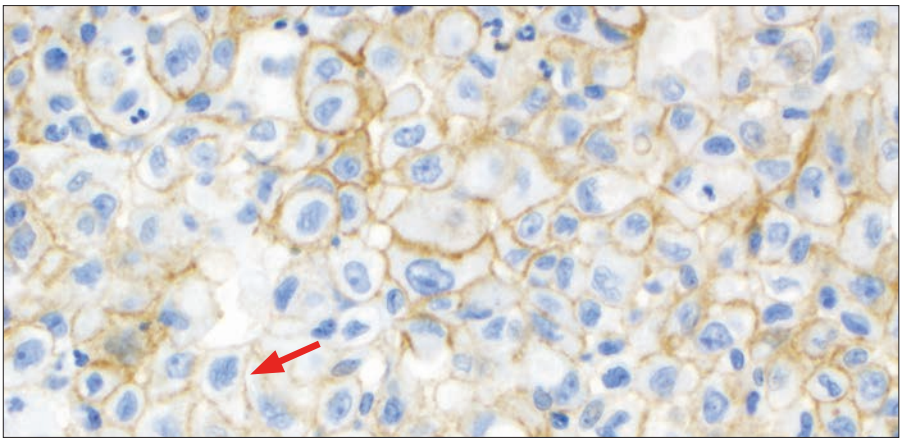


Figure 18b: NSCLC specimen stained with PD-L1 primary antibody exhibiting weak but perceptible and convincing membrane staining of tumor cells (arrow) (40× magnification).

Key Point

Any perceptible and convincing membrane staining of tumor cells (≥ 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+.

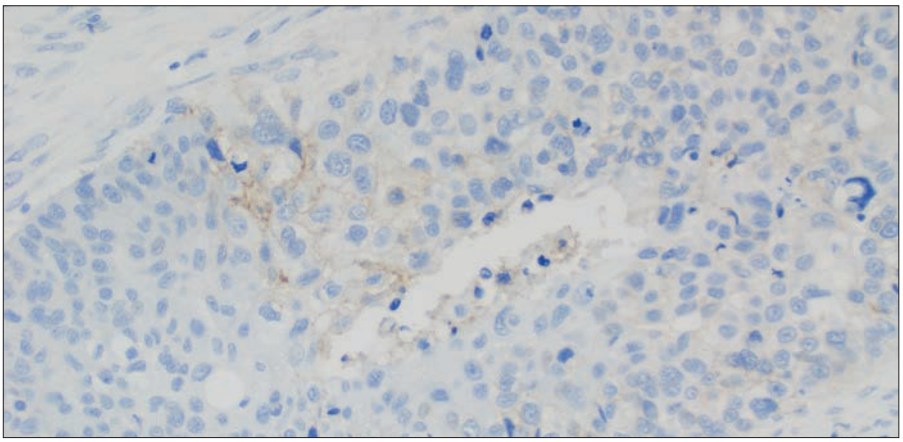


Figure 19a: NSCLC specimen stained with PD-L1 primary antibody exhibiting weak but perceptible and convincing membrane staining of tumor cells (20× magnification).

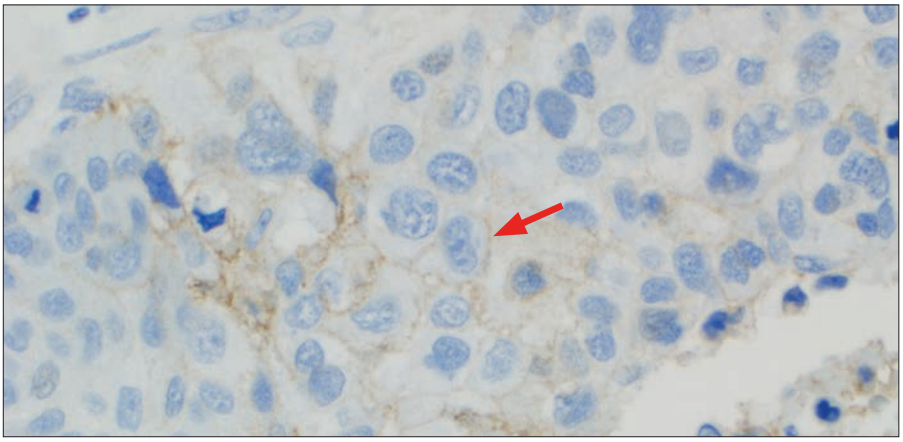


Figure 19b: NSCLC specimen stained with PD-L1 primary antibody exhibiting weak but perceptible and convincing membrane staining of tumor cells (arrow) (40× magnification).

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 (≥ 1+). Tumor-associated immune cells should be excluded from scoring.

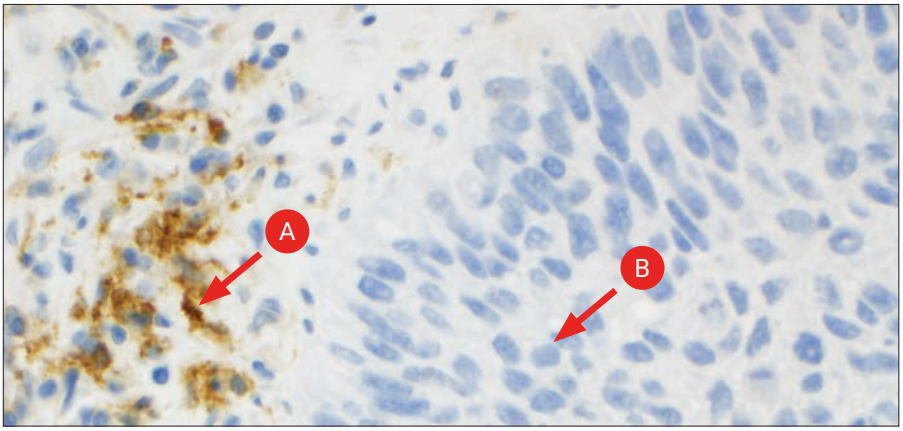


Figure 20: NSCLC specimen stained with PD-L1 primary antibody exhibiting strong staining of the TAIC (A) and lack of PD-L1 staining of tumor cells (B); TAIC staining should be excluded from the scoring (20× magnification).

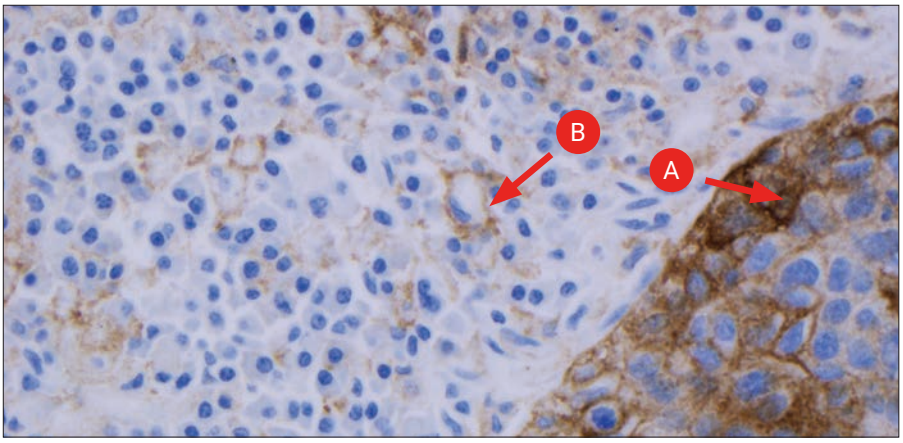


Figure 21: NSCLC specimen stained with PD-L1 primary antibody exhibiting strong staining of tumor cells (A) and moderate staining of the TAIC (B); TAIC staining should be excluded from the scoring (20× magnification).

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+).

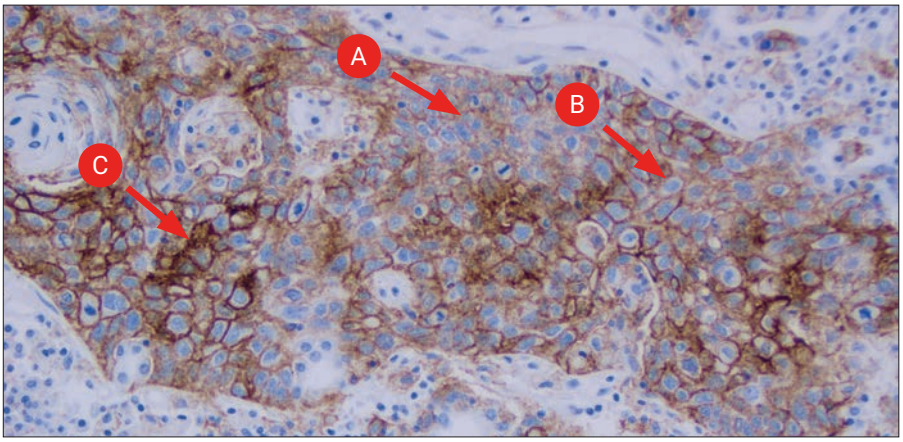


Figure 22: NSCLC specimen stained with PD-L1 primary antibody exhibiting a heterogeneous membrane staining pattern with various staining intensities: 1+ staining (A), 2+ staining (B), and 3+ staining (C) (20× magnification).

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 (≥ 1+).

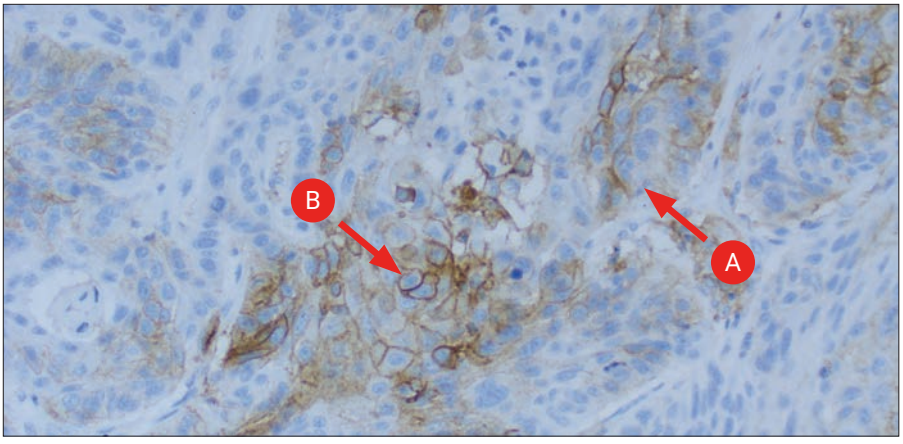


Figure 23: NSCLC specimen stained with PD-L1 primary antibody exhibiting a heterogeneous membrane staining pattern with various staining intensities (1–3+): partial membrane staining of tumor cell (A) and complete cell membrane staining (B) (20× magnification).

Key Point

Partial and/or complete membrane staining of tumor cells (≥ 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.

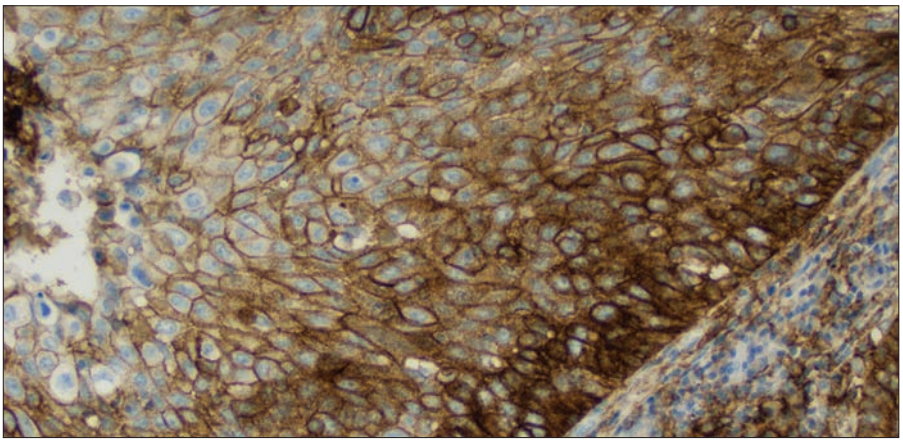


Figure 24: NSCLC specimen stained with PD-L1 primary antibody exhibiting strong cytoplasmic and membrane staining of tumor cells (20× magnification).

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.

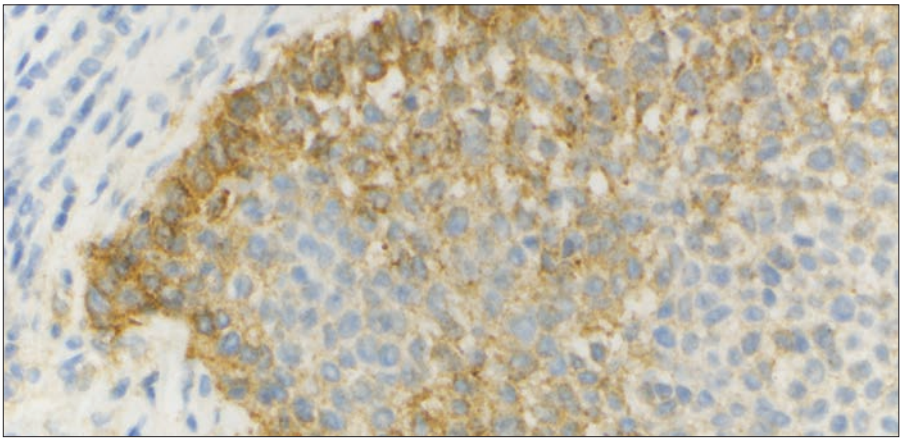


Figure 25: NSCLC specimen stained with PD-L1 primary antibody with the majority of tumor cells exhibiting a granular pattern of perceptible and convincing membrane staining (20× magnification).

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.

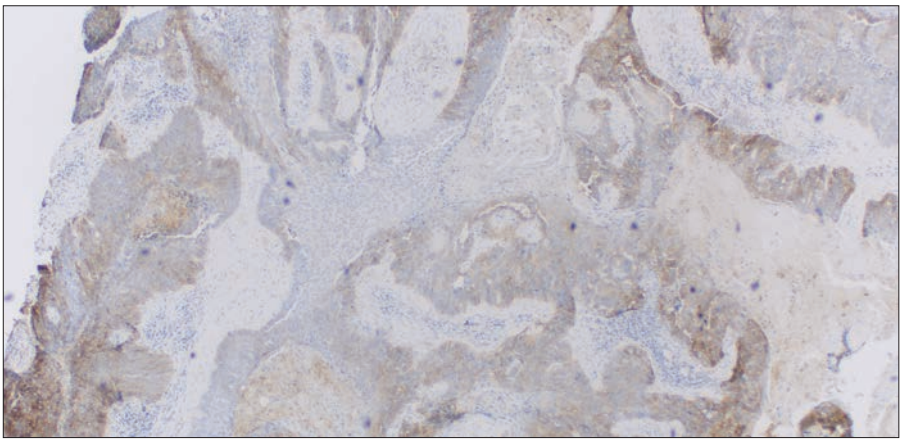


Figure 26: NSCLC specimen stained with PD-L1 primary antibody exhibiting a patchy membrane staining pattern (10× magnification).

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.

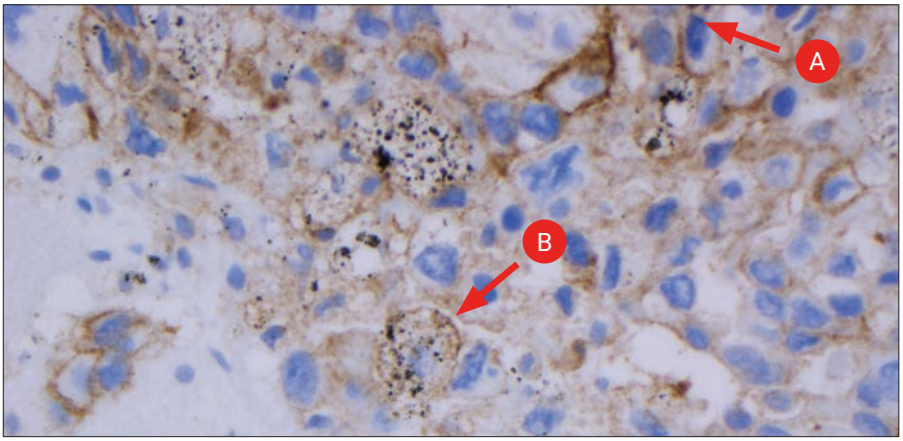


Figure 27: NSCLC specimen stained with PD-L1 primary antibody exhibiting strong staining of tumor cells (A) and moderate staining of the TAIC (B); TAIC staining should be excluded from the scoring (20× magnification).

Key Point

Anthracotic pigment should be disregarded

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

Case 1: TPS < 1%

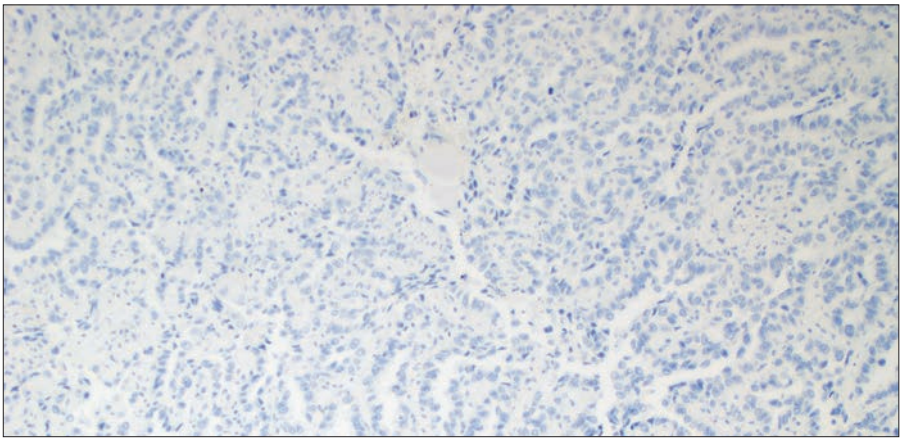


Figure 28a: 10× magnification.

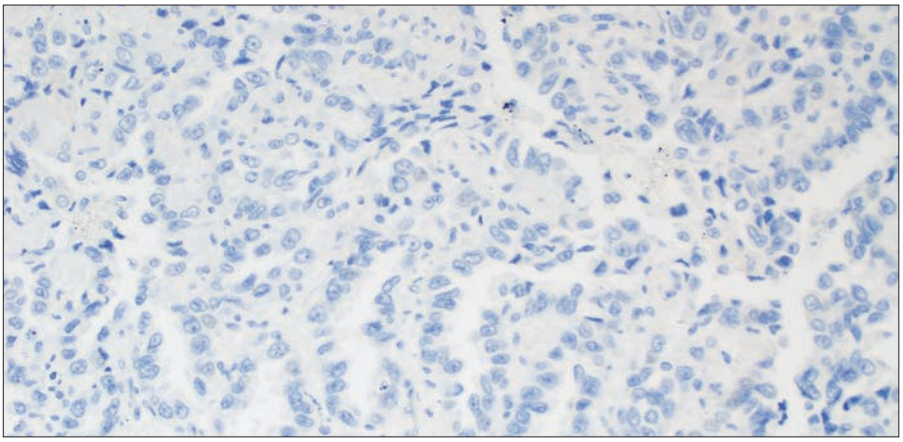


Figure 28b: 20× magnification.

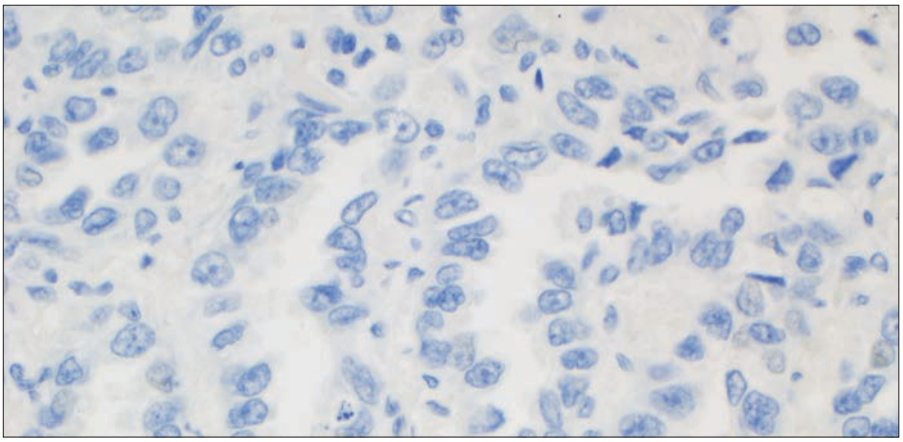


Figure 28c: 40× magnification.

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

Case 2: TPS < 1%

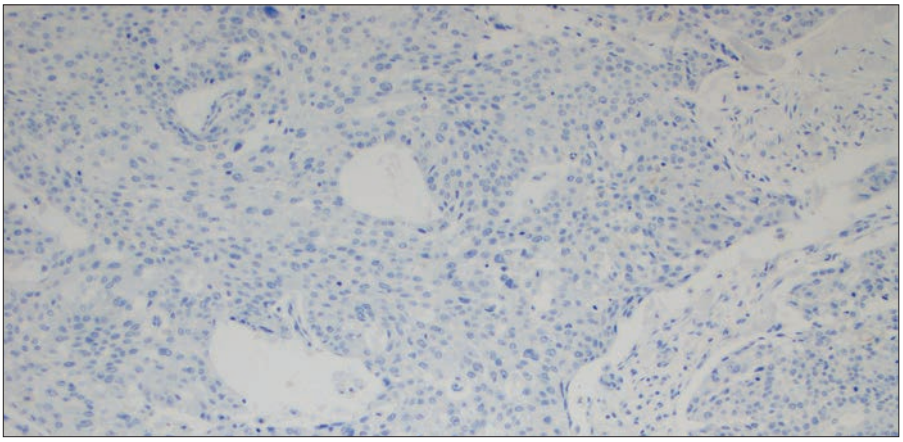


Figure 29a: 10× magnification.

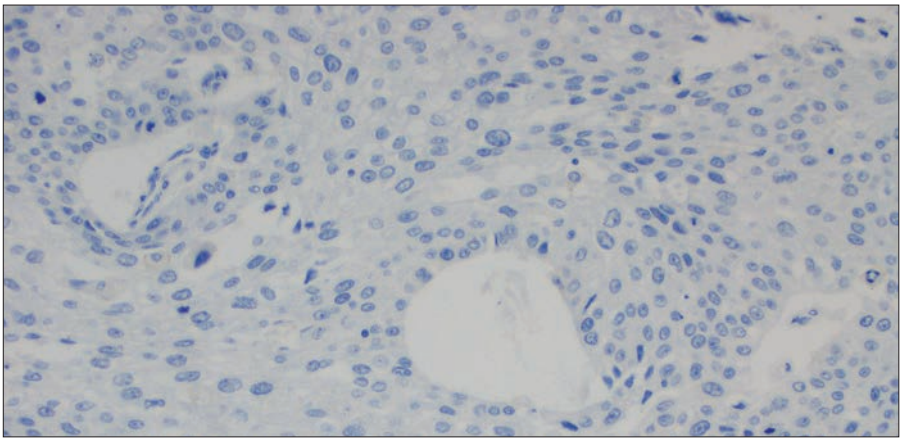


Figure 29b: 20× magnification.

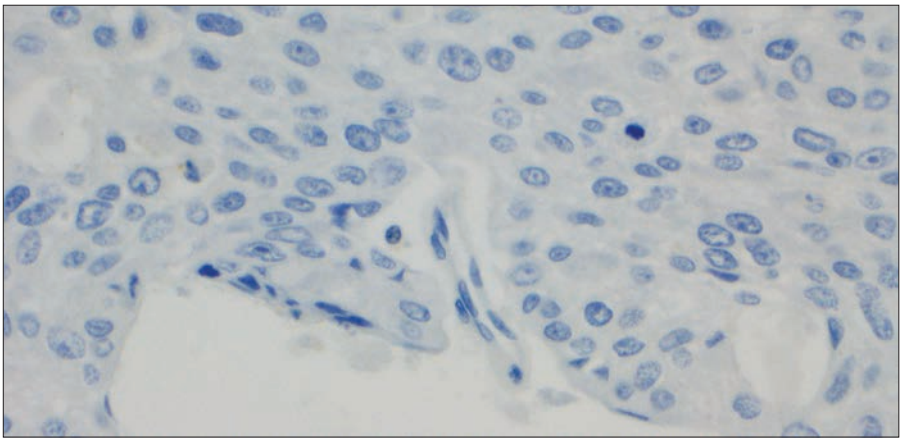


Figure 29c: 40× magnification.

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

Case 3: TPS < 1%

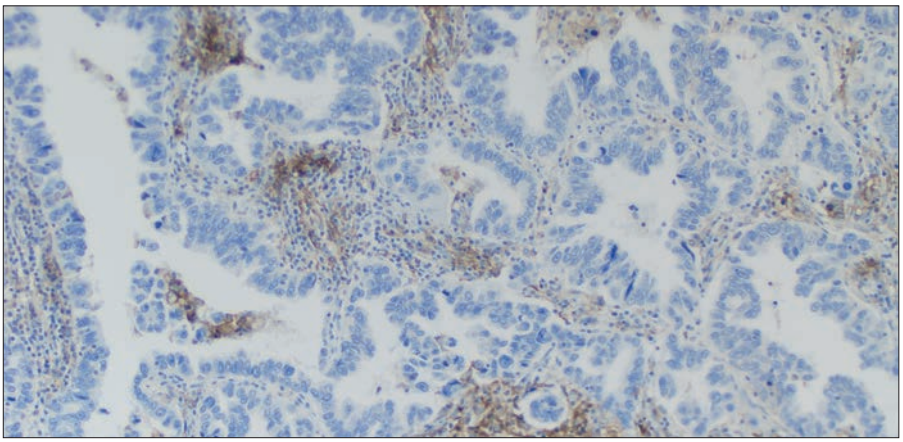


Figure 30a: 10× magnification.

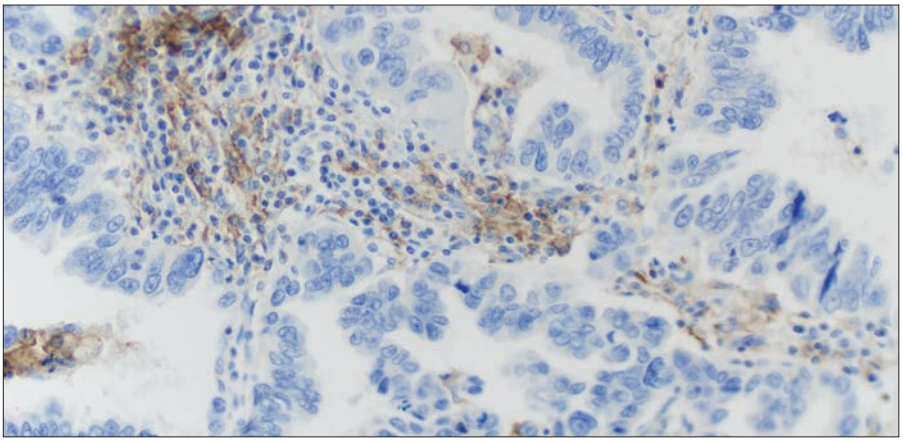


Figure 30b: 20× magnification.

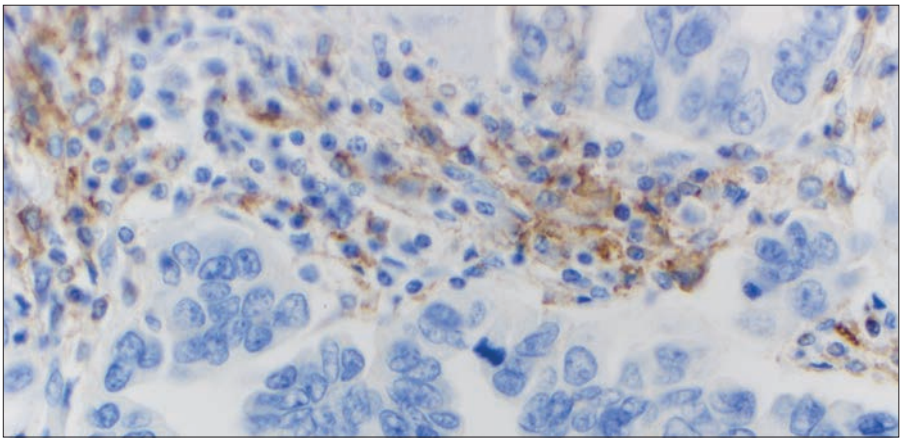


Figure 30c: 40× magnification.

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%

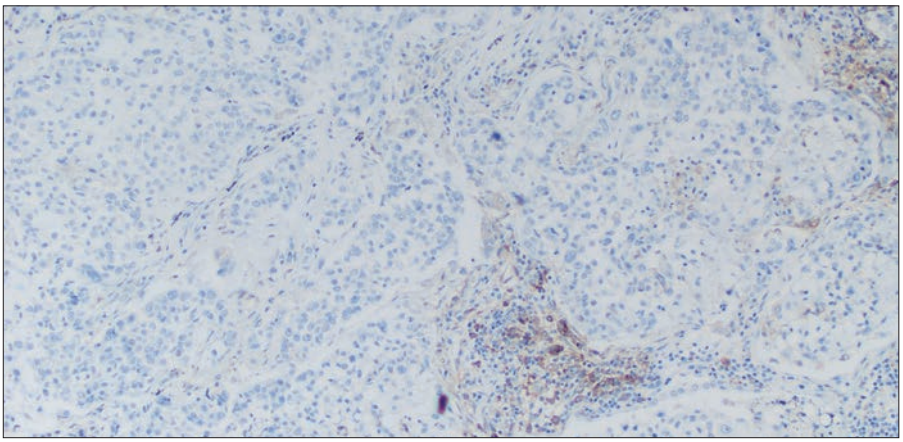


Figure 31a: 10× magnification.

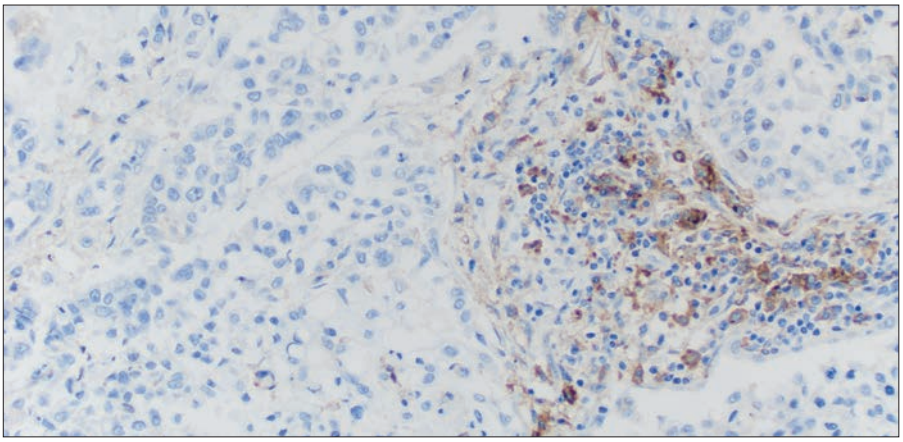


Figure 31b: 20× magnification.

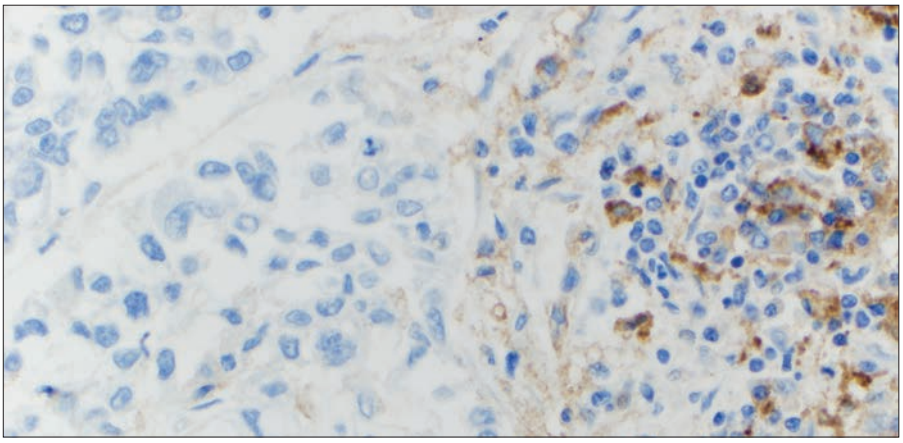


Figure 31c: 40× magnification.

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%

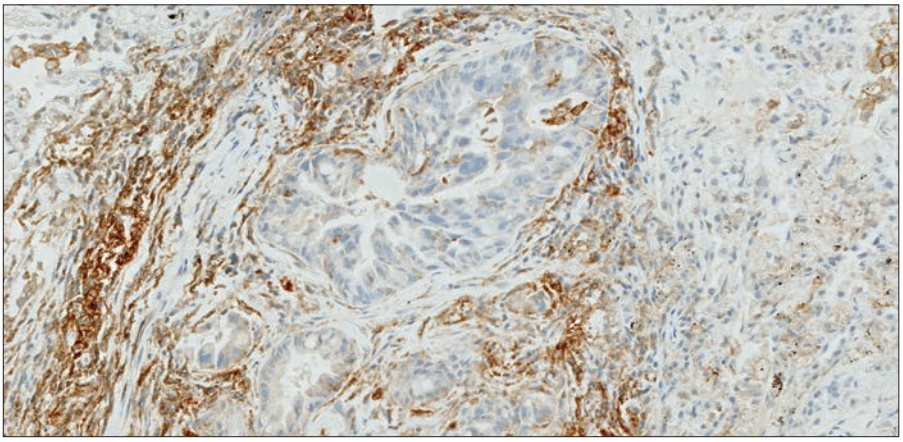


Figure 32a: 10× magnification.

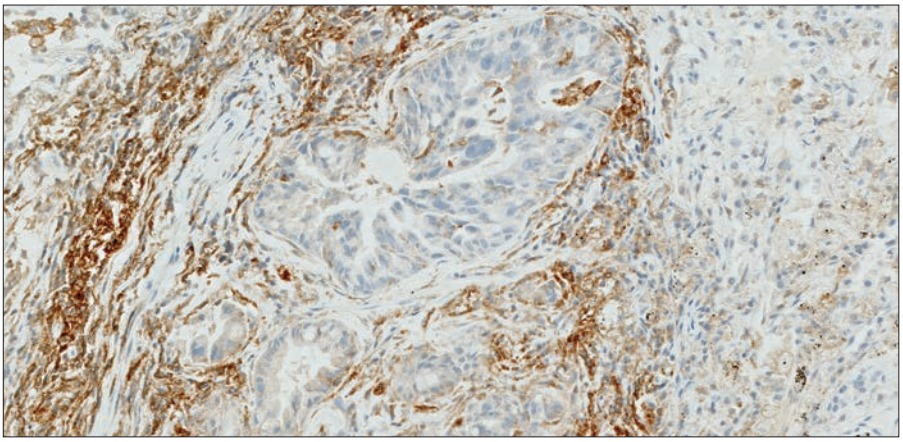


Figure 32b: 20× magnification.

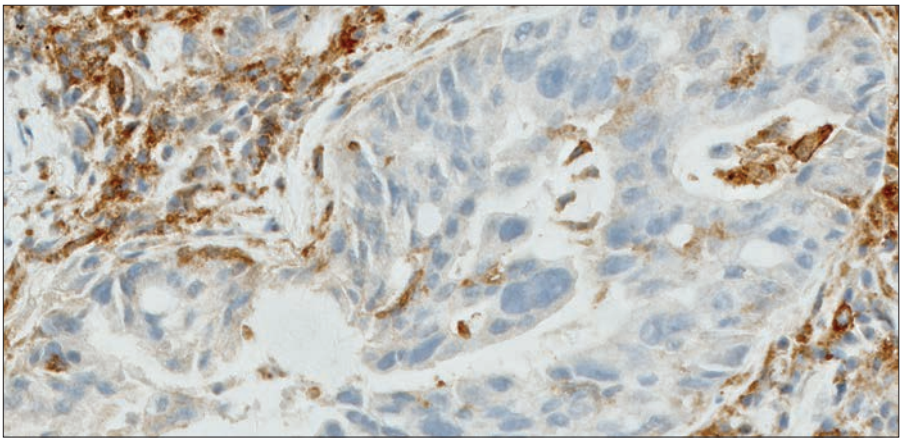


Figure 32c: 40× magnification.

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

Challenging Case 2: TPS 0–10%

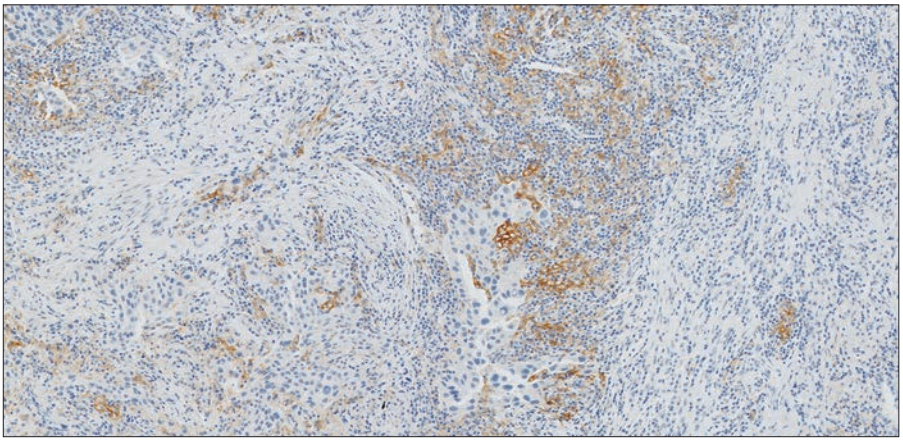


Figure 33a: 10× magnification.

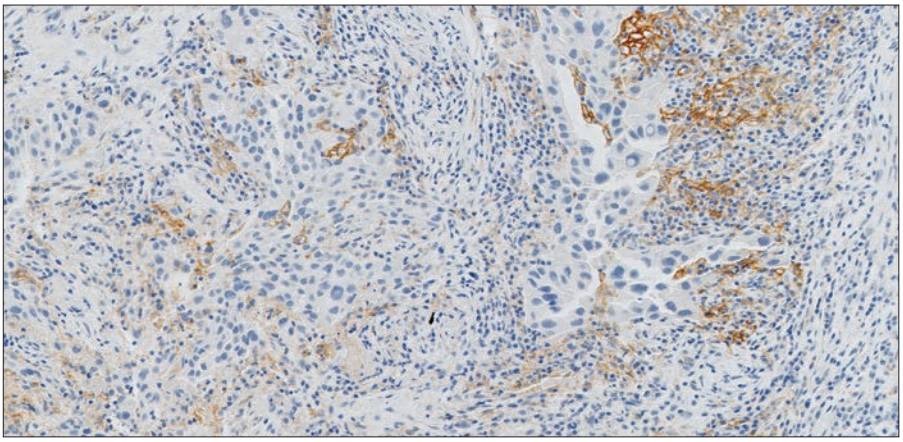


Figure 33b: 20× magnification.

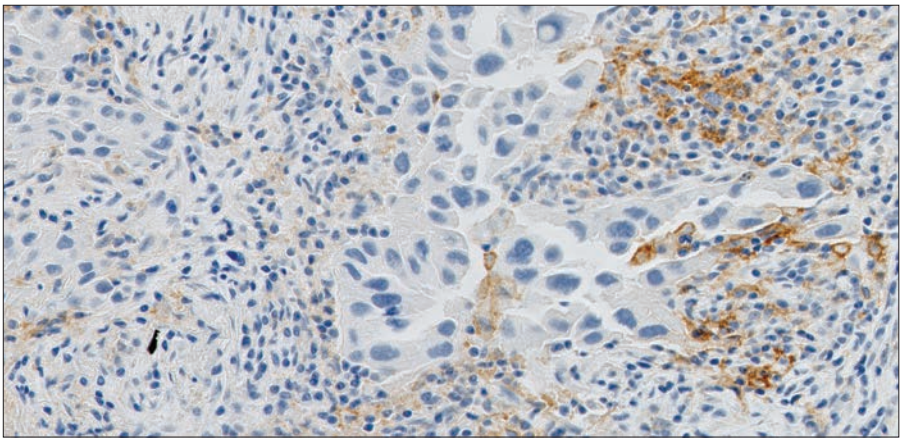


Figure 33c: 40× magnification.

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

Challenging Case 3: TPS 0–10%

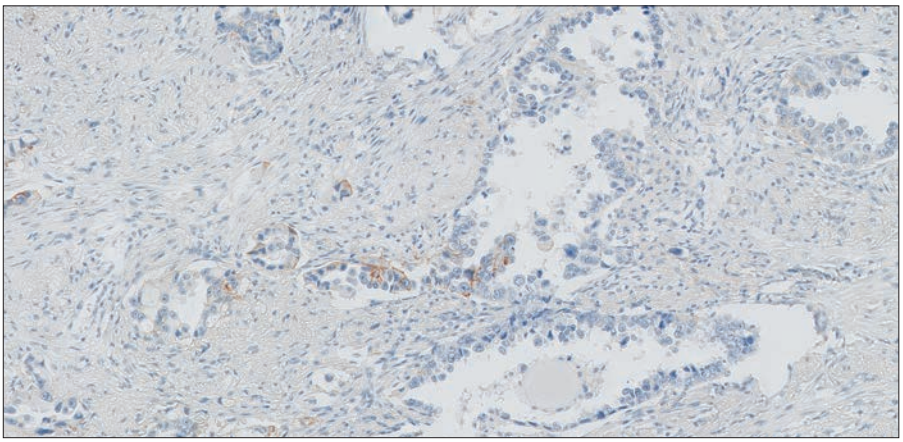


Figure 34a: 10× magnification.

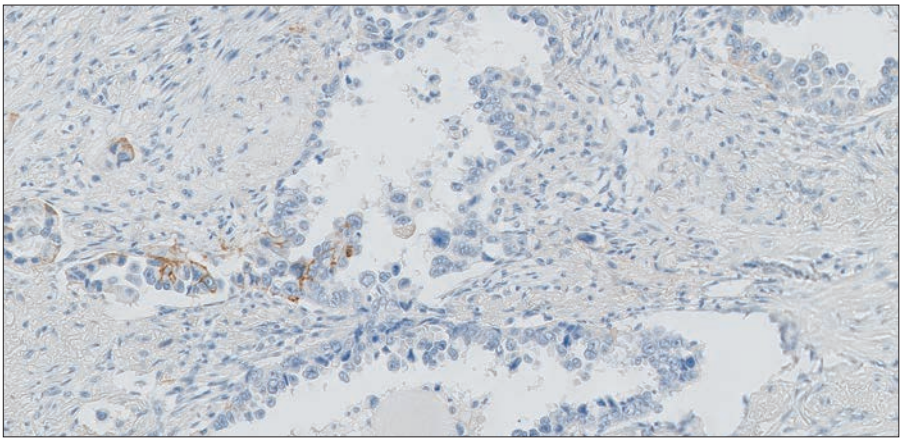


Figure 34b: 20× magnification.

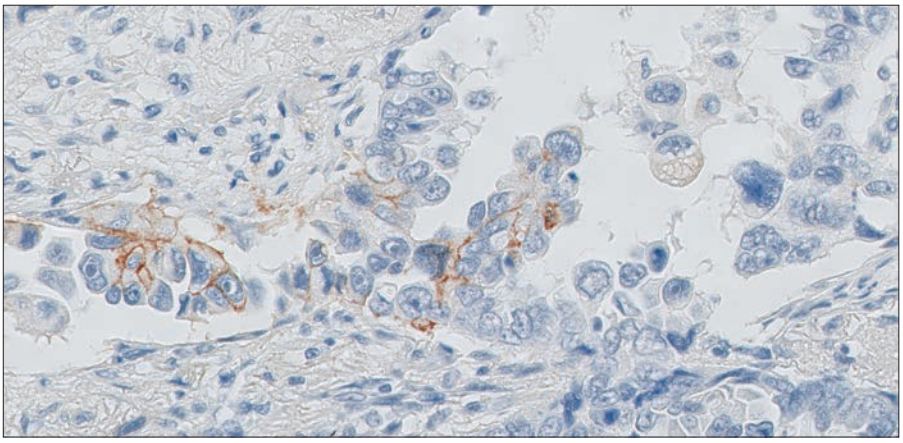


Figure 34c: 40× magnification.

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

Challenging Case 4: TPS 0–10%

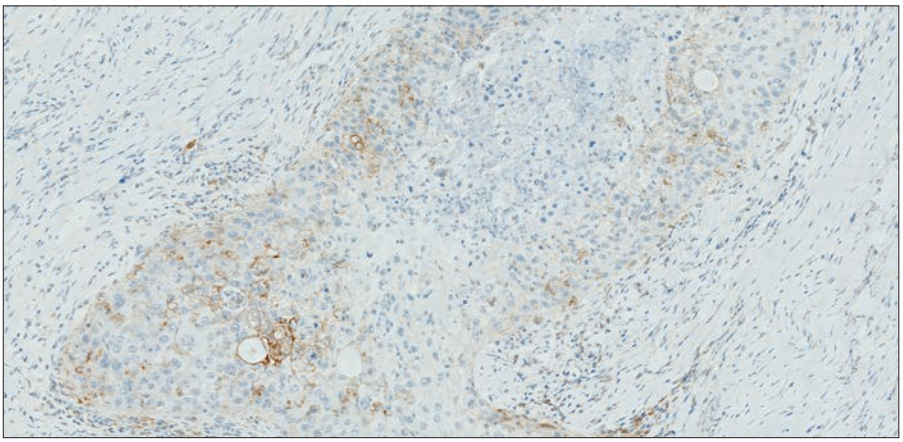


Figure 35a: 10× magnification.

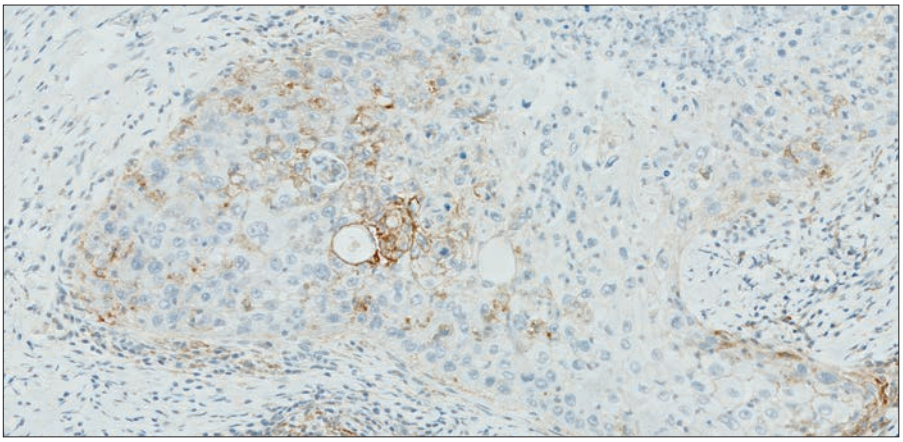


Figure 35b: 20× magnification.

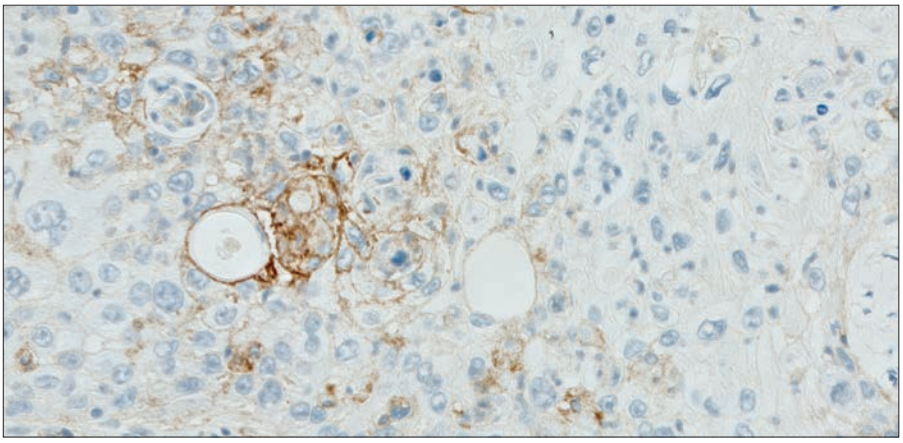


Figure 35c: 40× magnification.

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

Challenging Case 5: TPS 0–10%

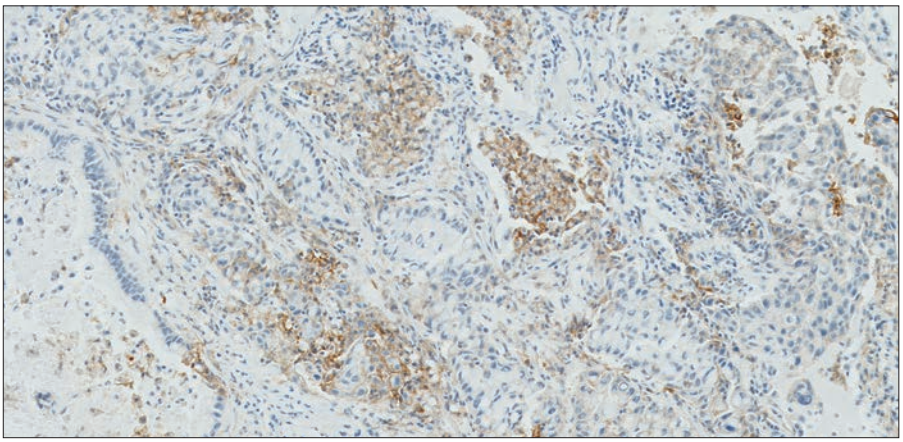


Figure 36a: 10× magnification.

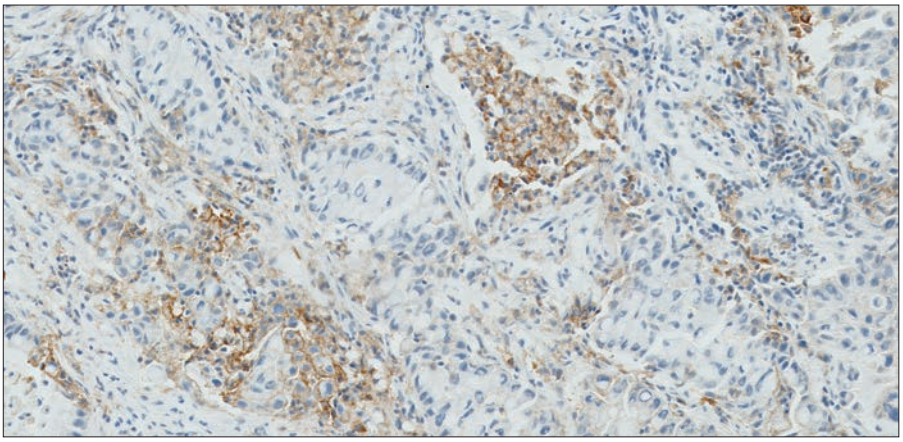


Figure 36b: 20× magnification.

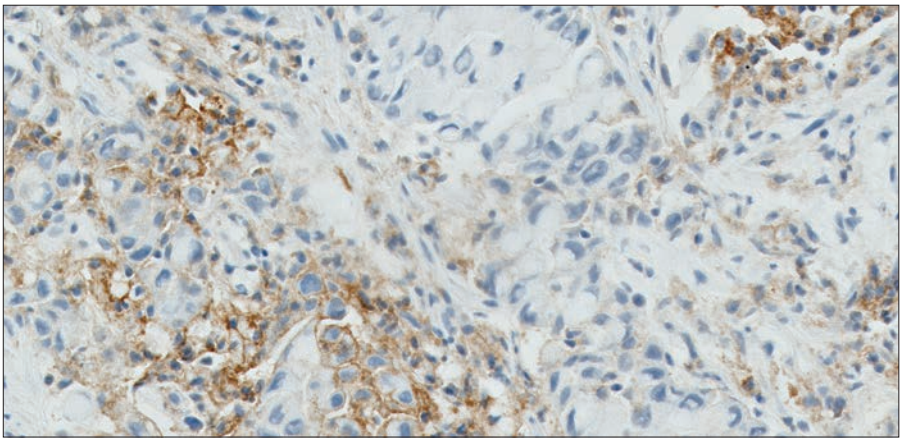


Figure 36c: 40× magnification.

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%

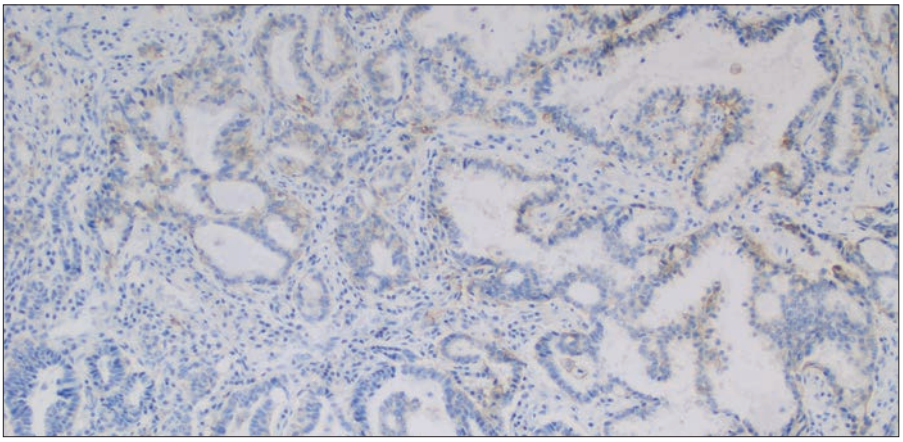


Figure 37a: 10× magnification.

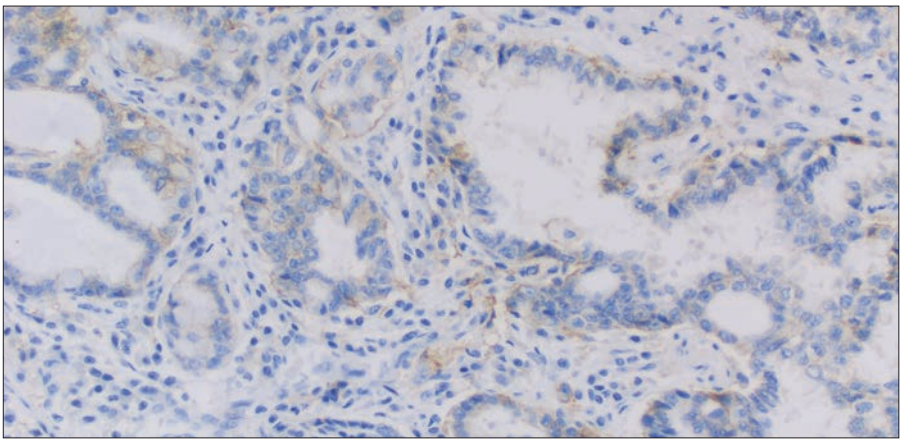


Figure 37b: 20× magnification.

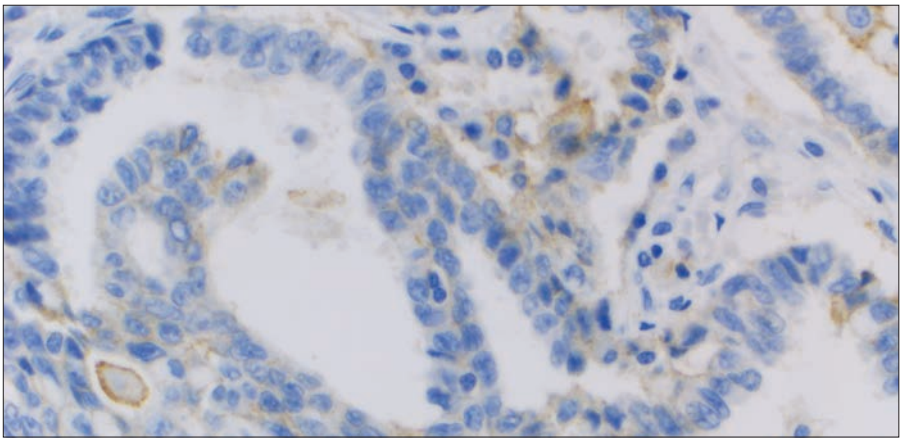


Figure 37c: 40× magnification.

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

Case 6: TPS 1–49%

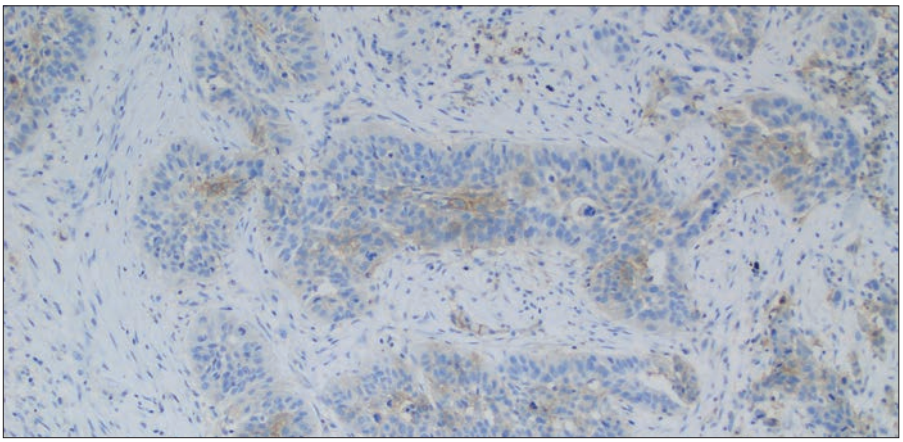


Figure 38a: 10× magnification.

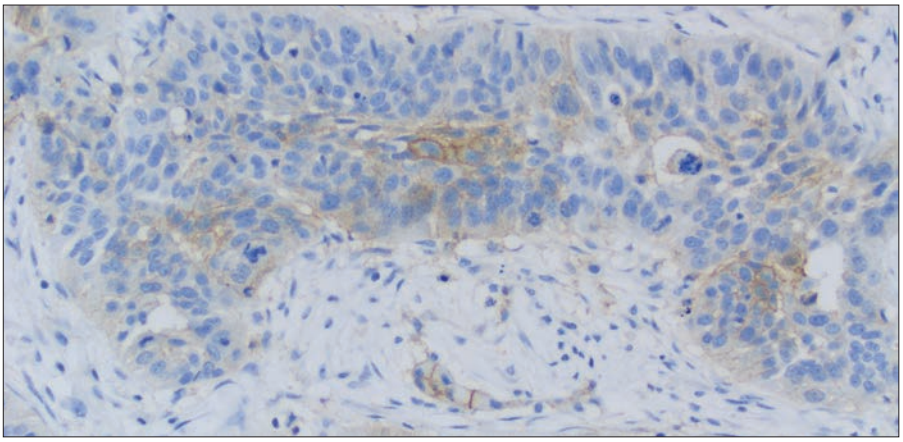


Figure 38b: 20× magnification.

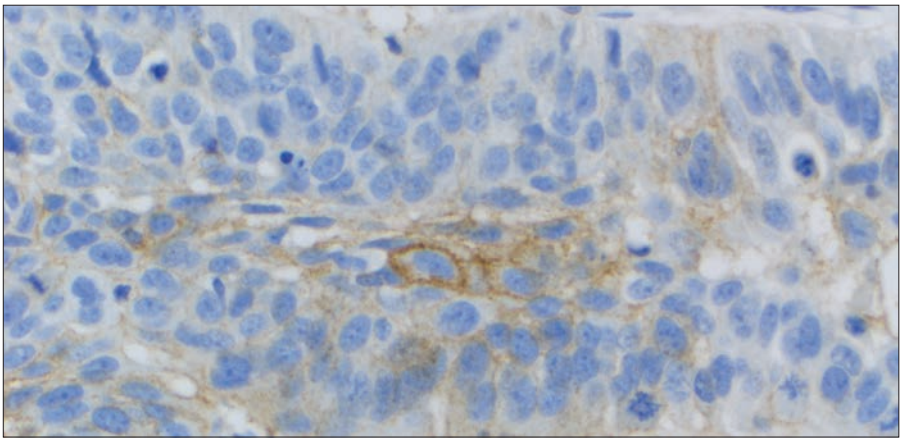


Figure 38c: 40× magnification.

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

Case 7: TPS 1–49%

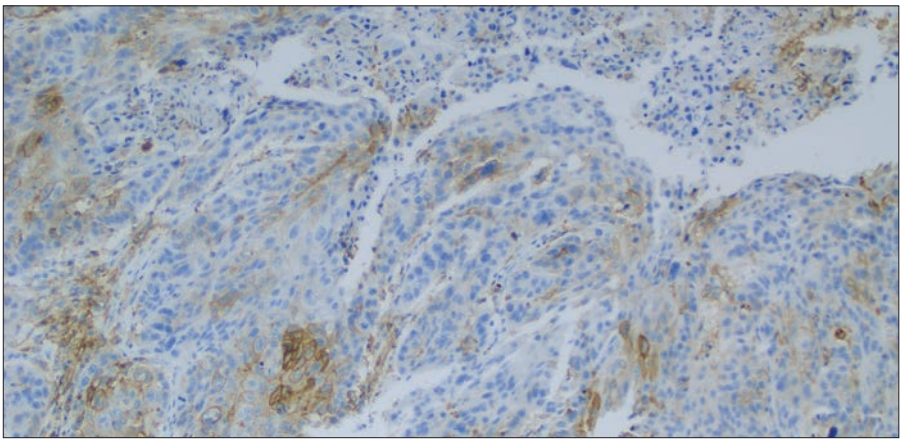


Figure 39a: 10× magnification.

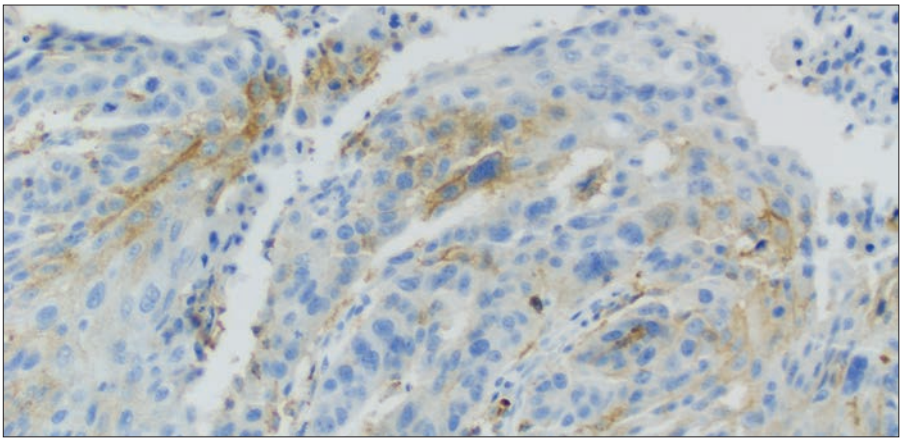


Figure 39b: 20× magnification.

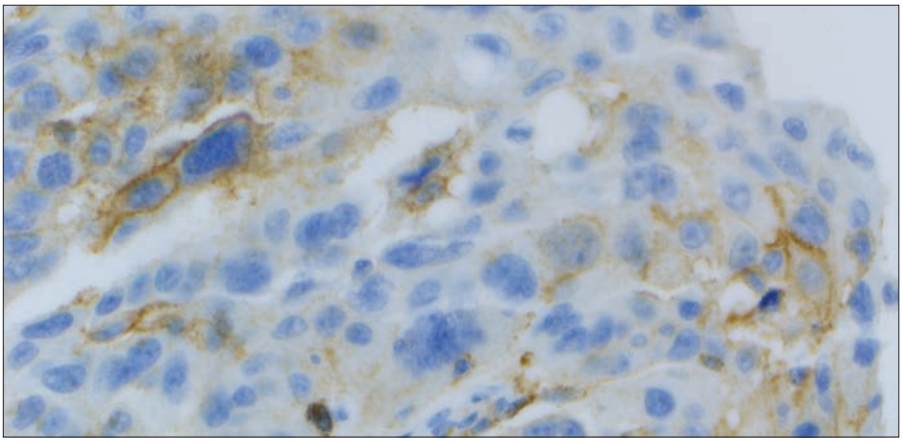


Figure 39c: 40× magnification.

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

Case 8: TPS 1–49%

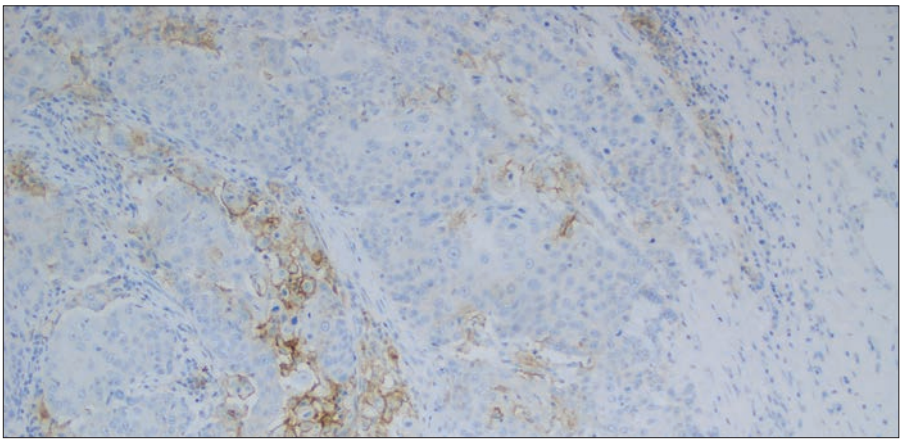


Figure 40a: 10× magnification.

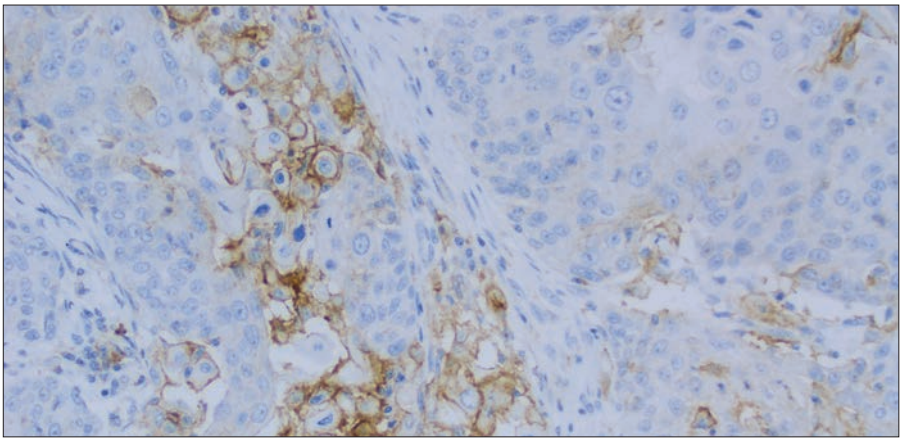


Figure 40b: 20× magnification.

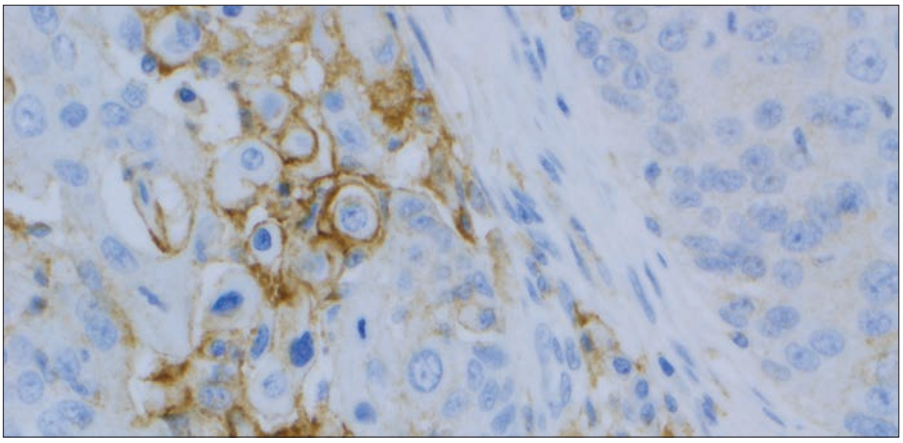


Figure 40c: 40× magnification.

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

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

Case 9: TPS ≥ 50%

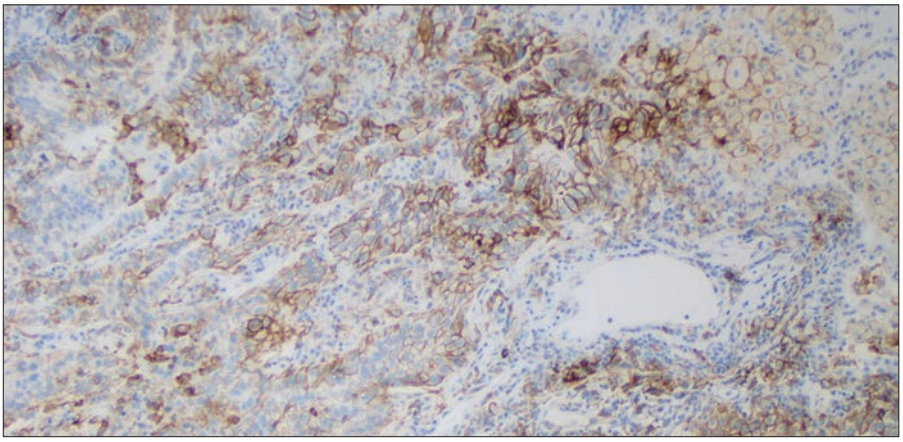


Figure 41a: 10× magnification.

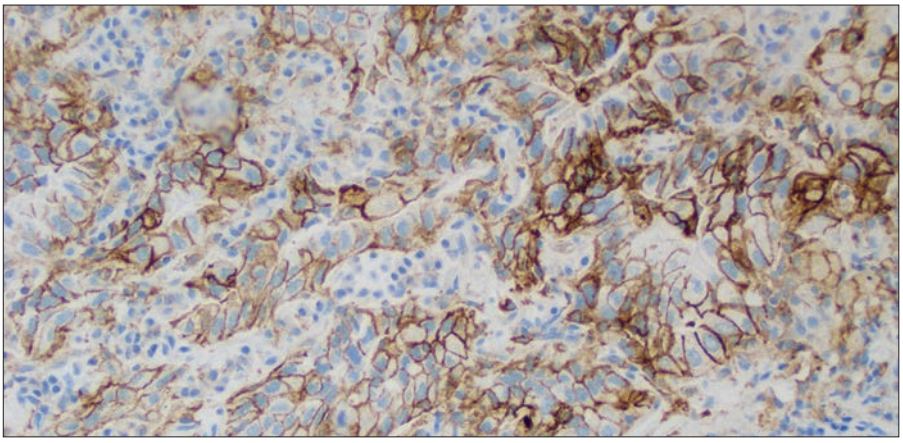


Figure 41b: 20× magnification.

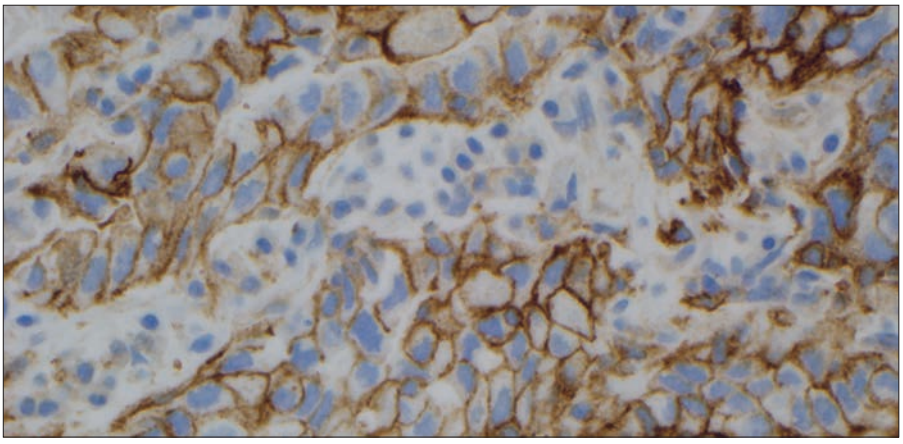


Figure 41c: 40× magnification.

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

Case 10: TPS ≥ 50%

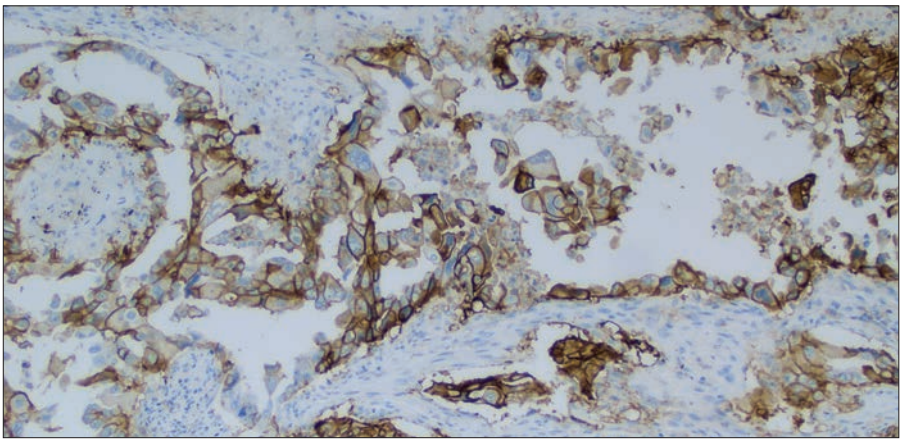


Figure 42a: 10× magnification.

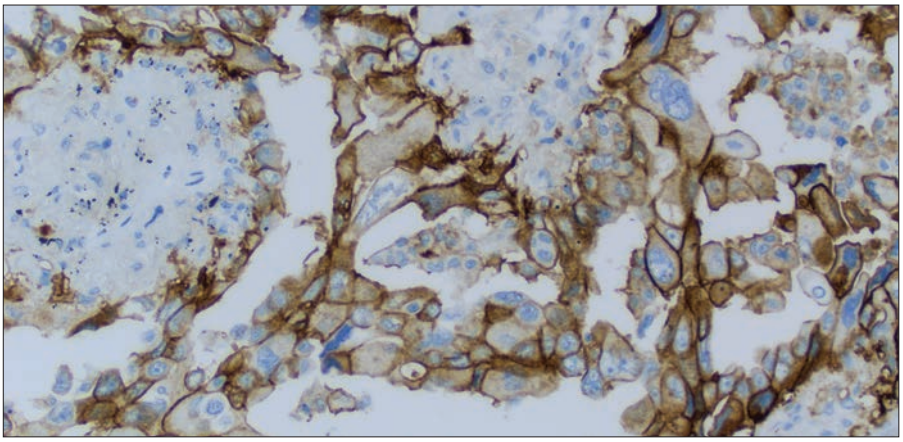


Figure 42b: 20× magnification.

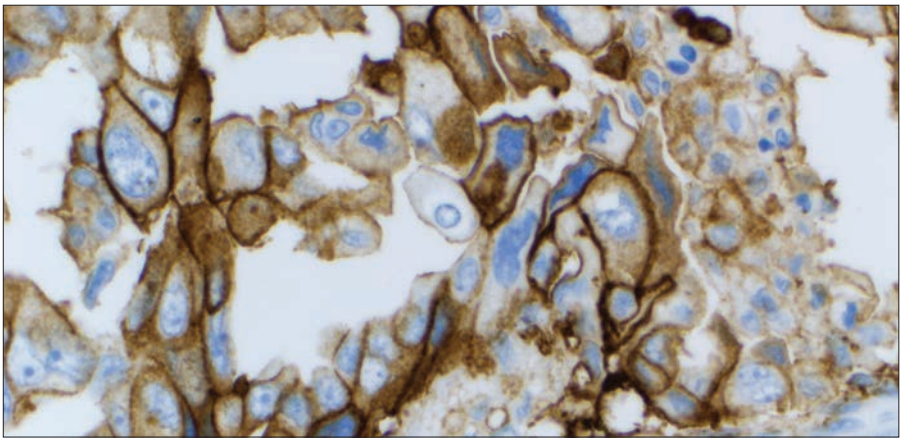


Figure 42c: 40× magnification.

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

Case 11: TPS ≥ 50%

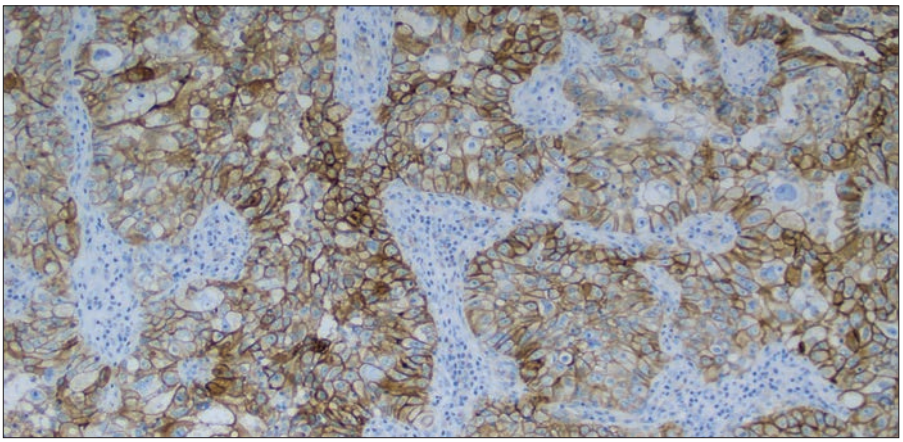


Figure 43a: 10× magnification.

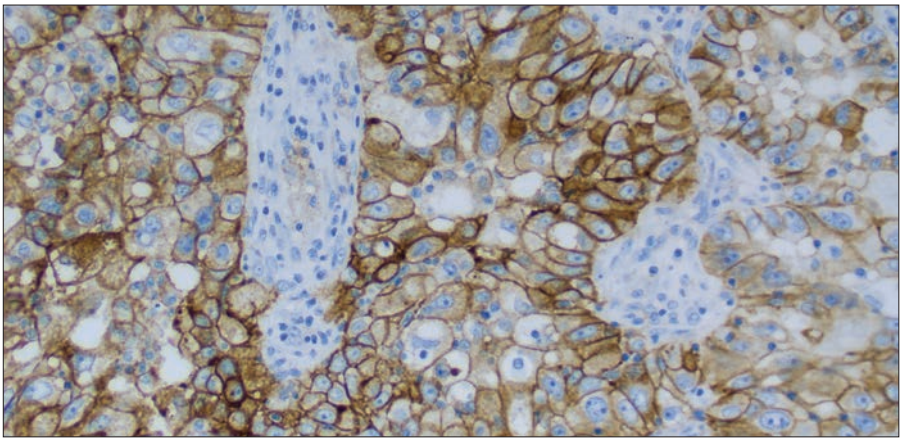


Figure 43b: 20× magnification.

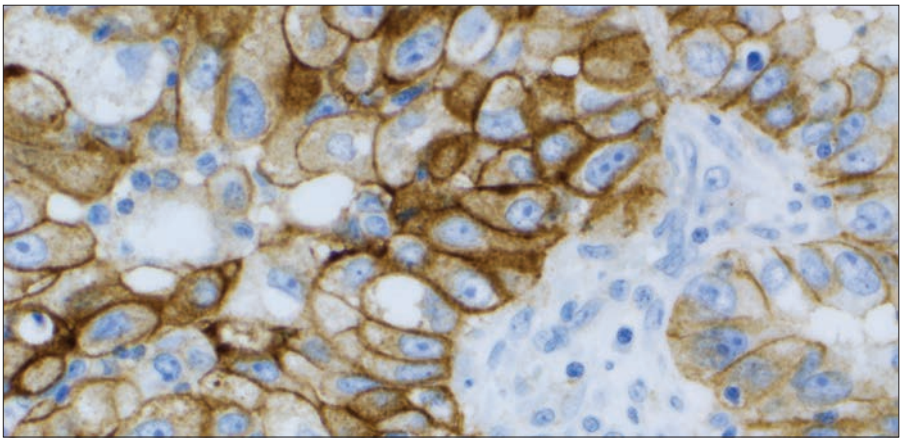


Figure 43c: 40× magnification.

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

Case 12: TPS ≥ 50%

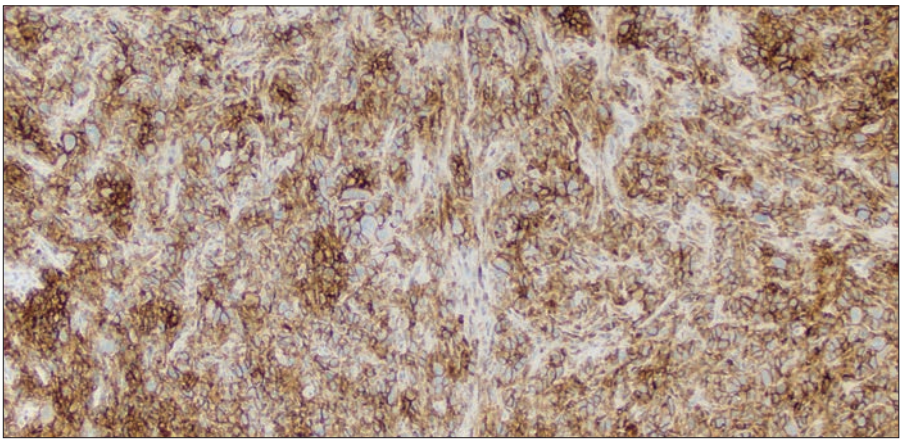


Figure 44a: 10× magnification.

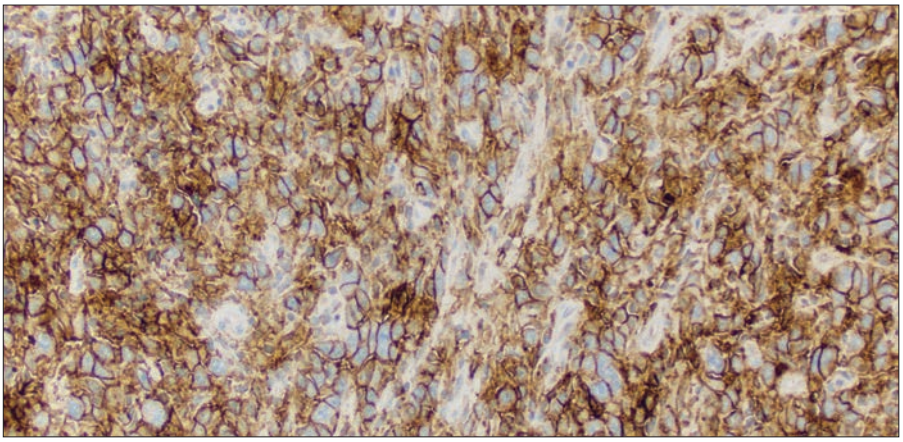


Figure 44b: 20× magnification.

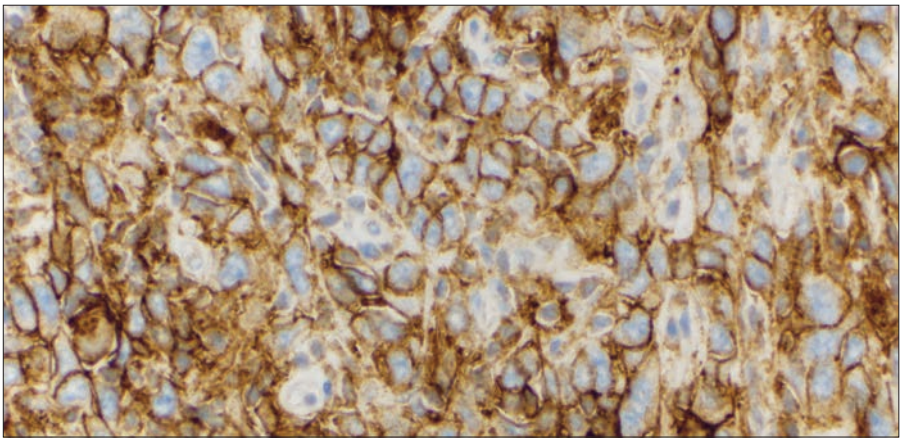


Figure 44c: 40× magnification.

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

Case 13: TPS ≥ 50%

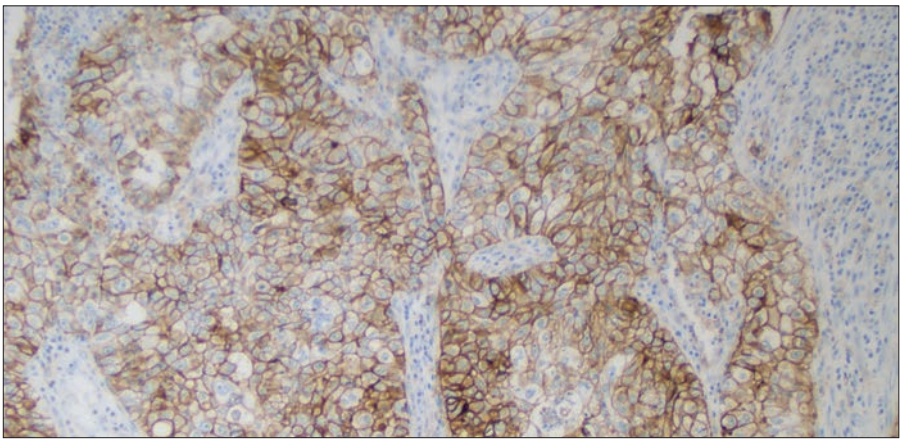


Figure 45a: 10× magnification.

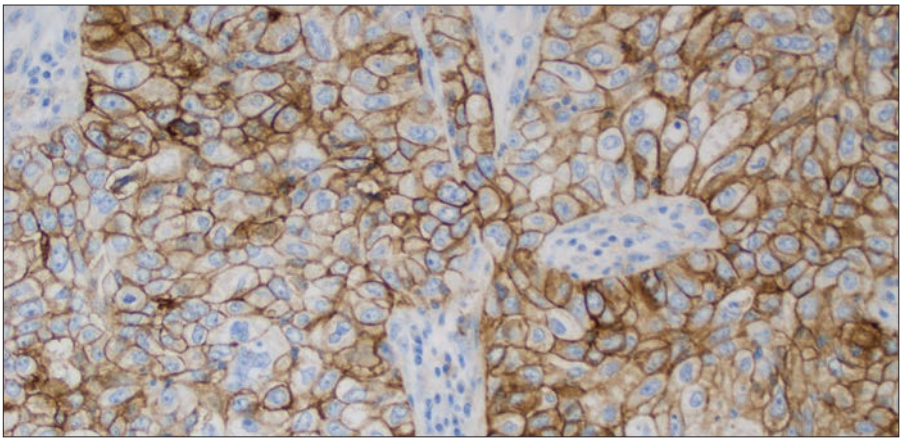


Figure 45b: 20× magnification.

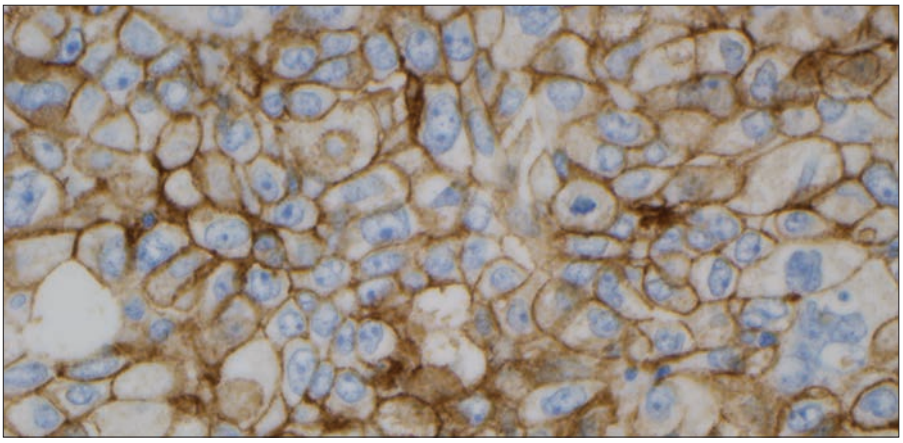


Figure 45c: 40× magnification.

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

Case 14: TPS ≥ 50%

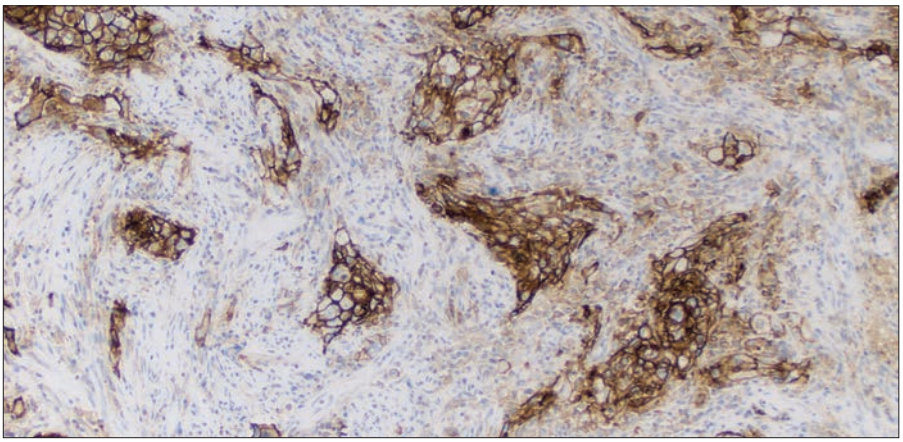


Figure 46a: 10× magnification.

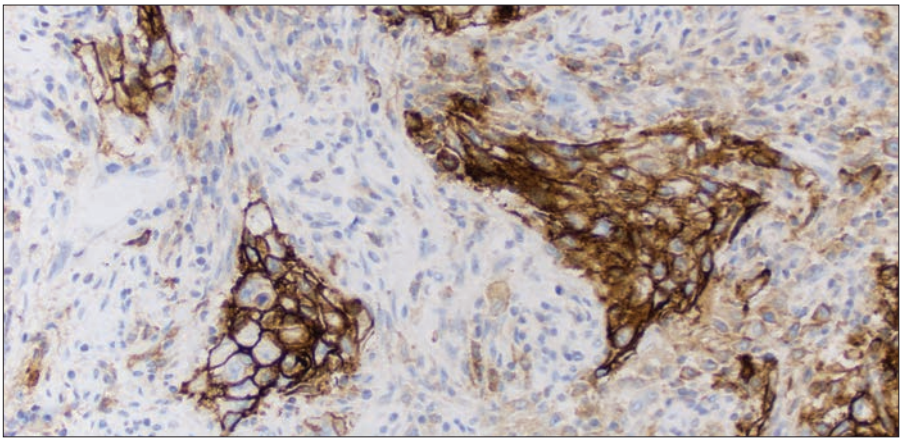


Figure 46b: 20× magnification.

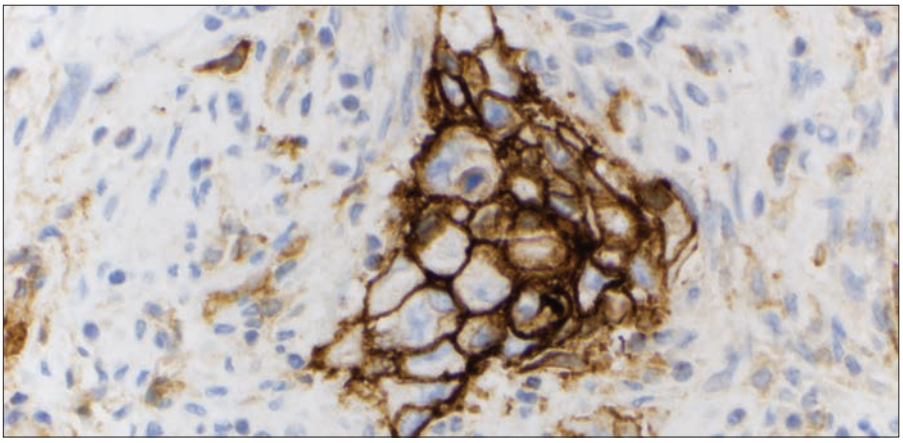


Figure 46c: 40× magnification.

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

Case 15: TPS ≥ 50%

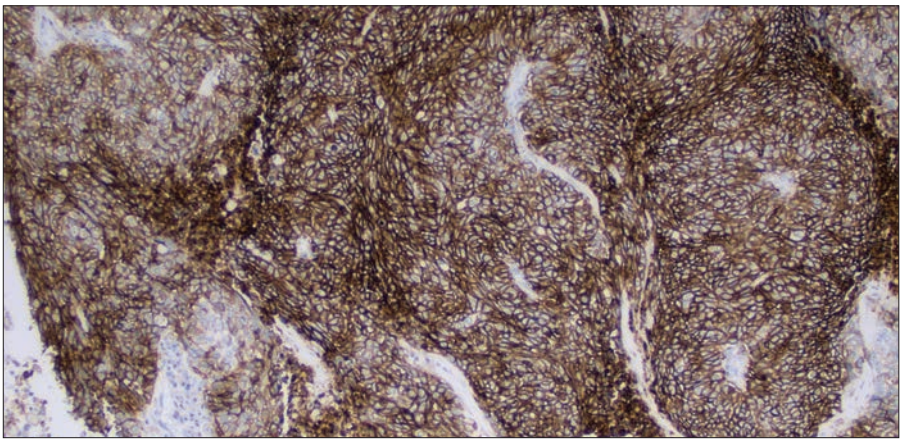


Figure 47a: 10× magnification.

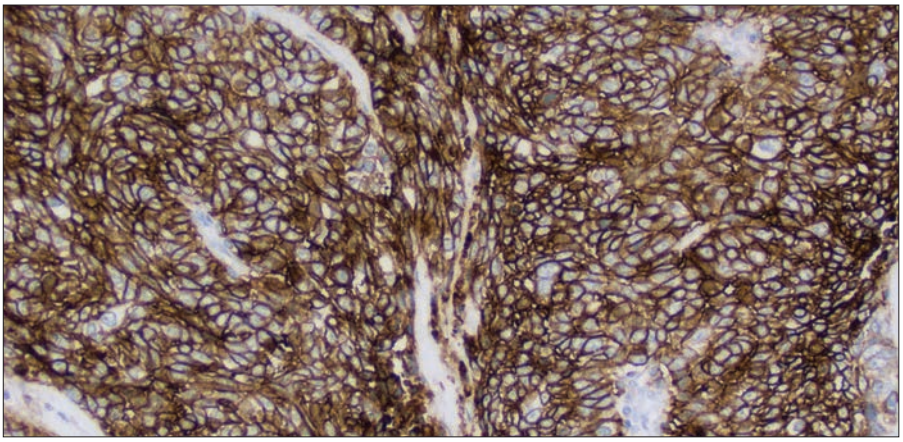


Figure 47b: 20× magnification.

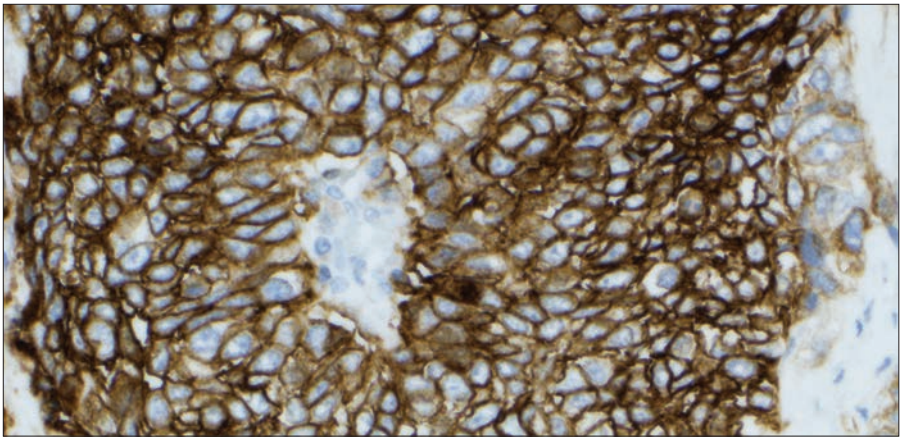


Figure 47c: 40× magnification.

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

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

Challenging Case 6: TPS 40–60%

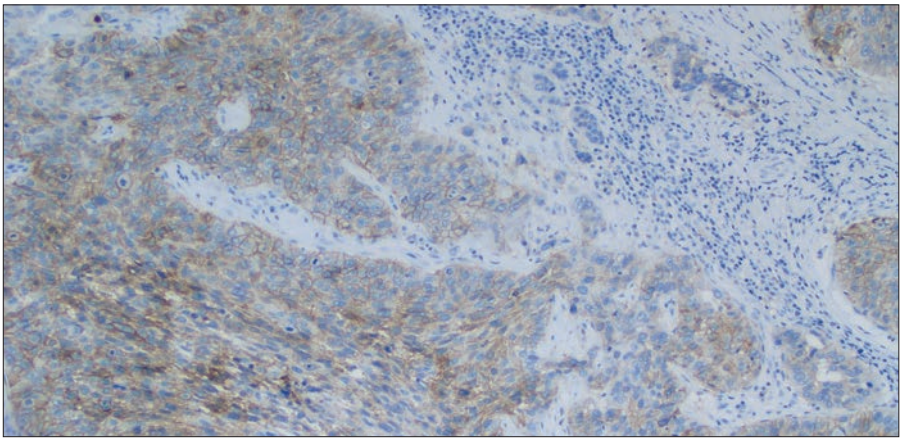


Figure 48a: 10× magnification.

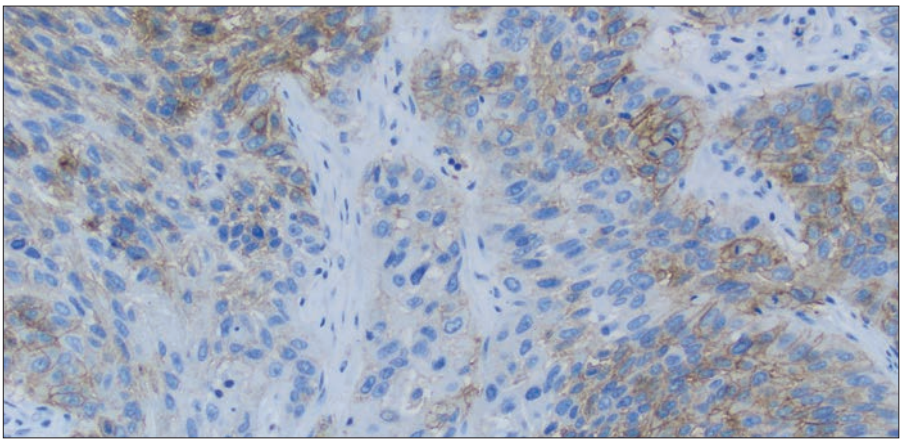


Figure 48b: 20× magnification.

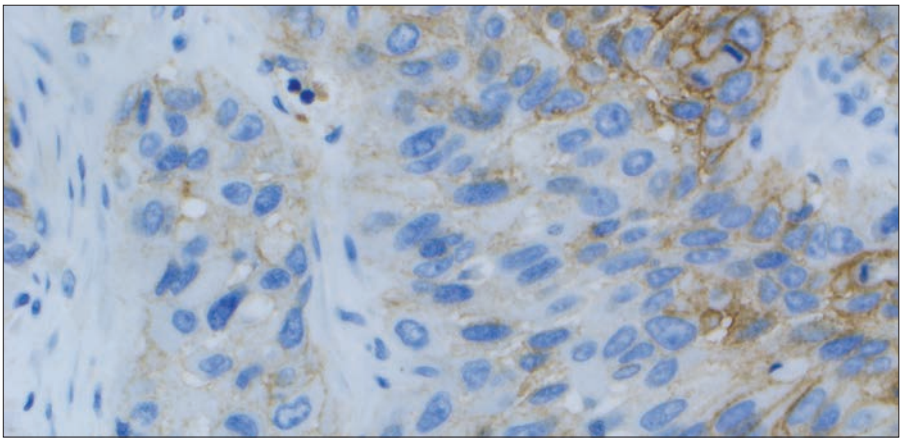


Figure 48c: 40× magnification.

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

Challenging Case 7: TPS 40–60%

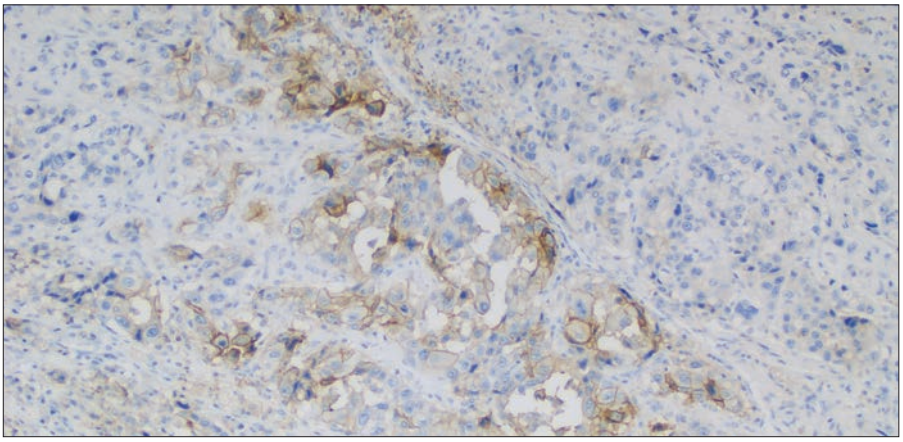


Figure 49a: 10× magnification.

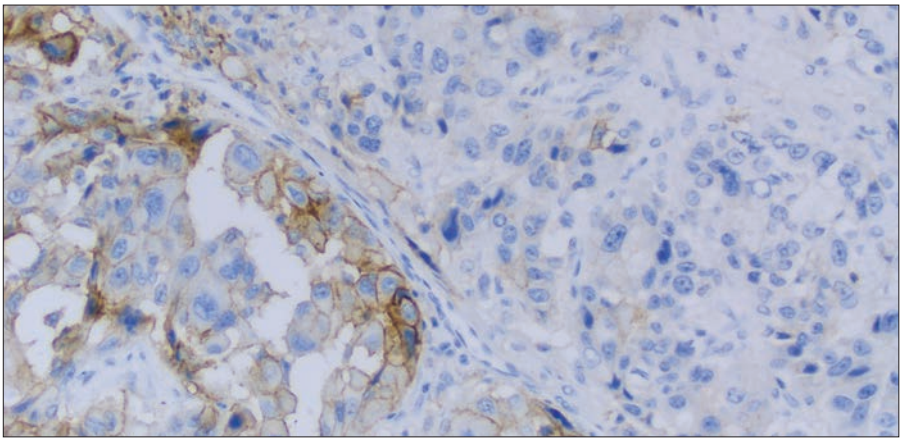


Figure 49b: 20× magnification.

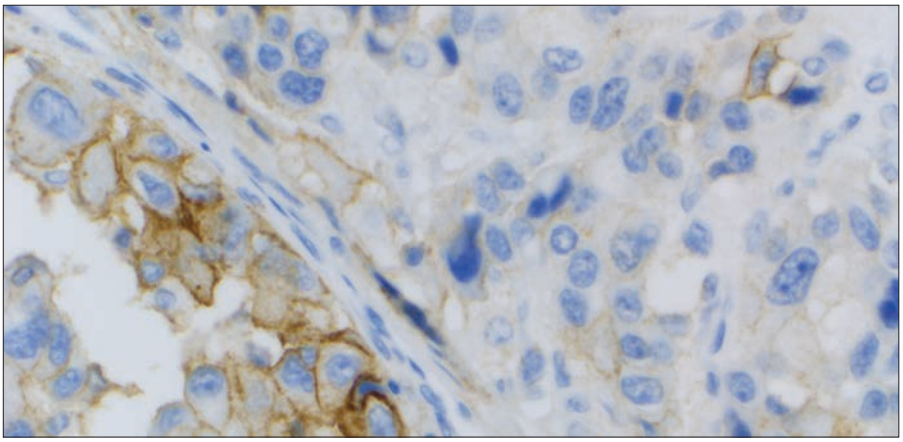


Figure 49c: 40× magnification.

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

Challenging Case 8: TPS 40–60%

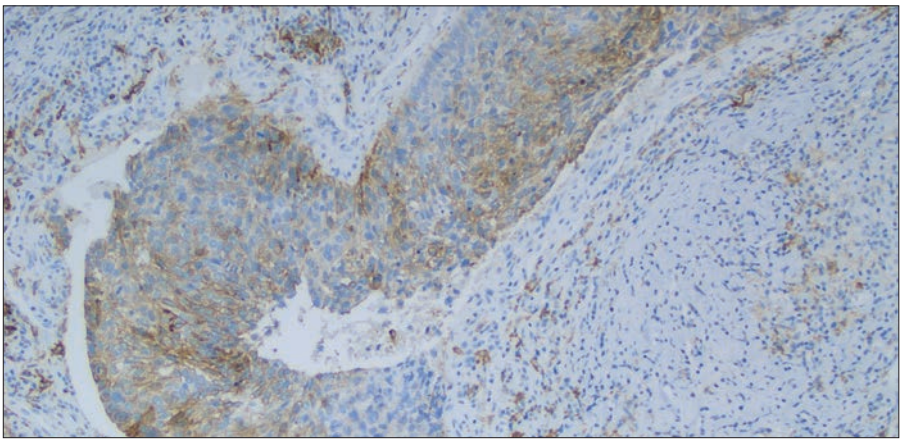


Figure 50a: 10× magnification.

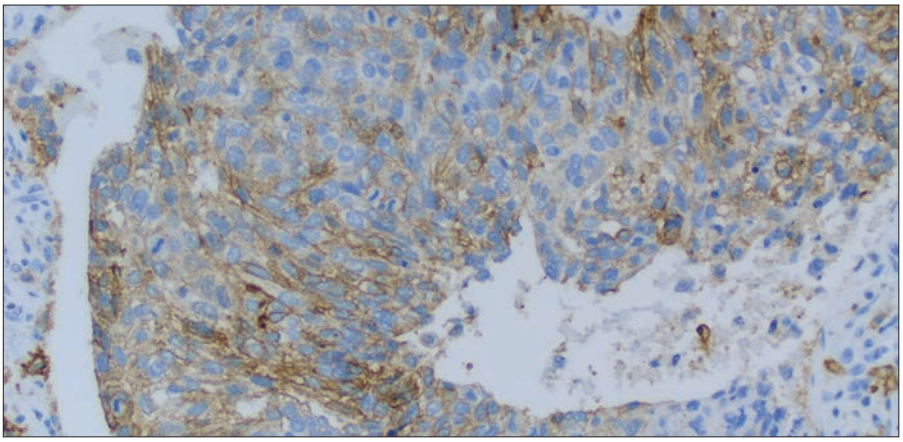


Figure 50b: 20× magnification.

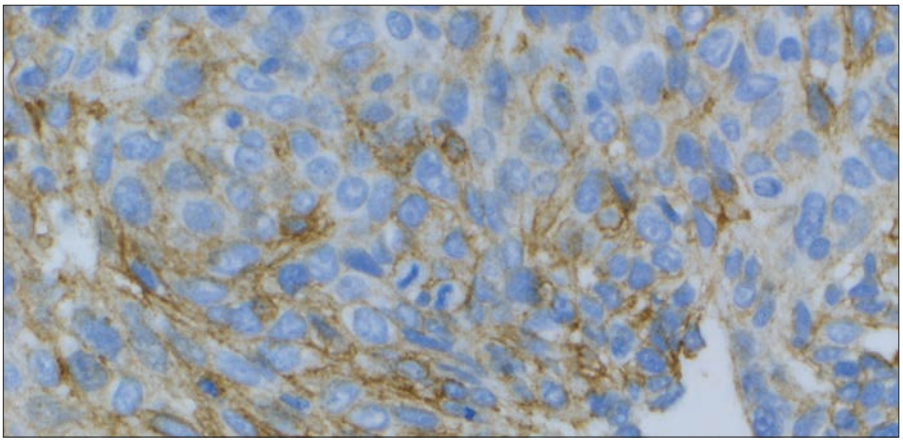


Figure 50c: 40× magnification.

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

Challenging Case 9: TPS 40–60%

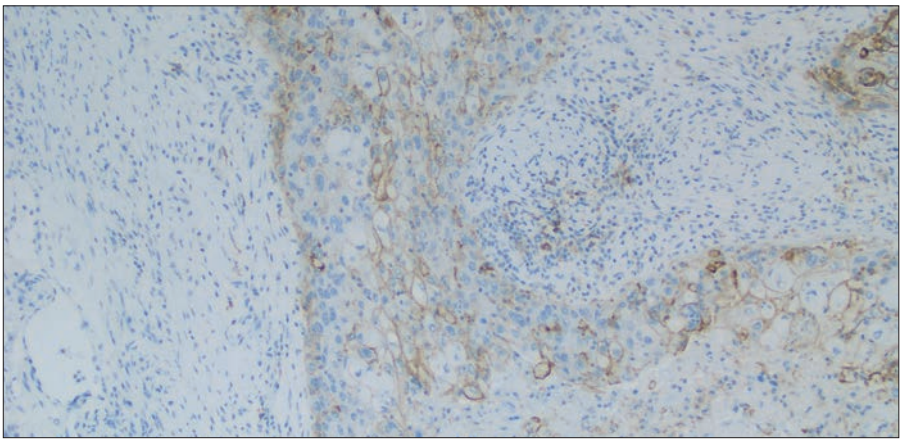


Figure 51a: 10× magnification.

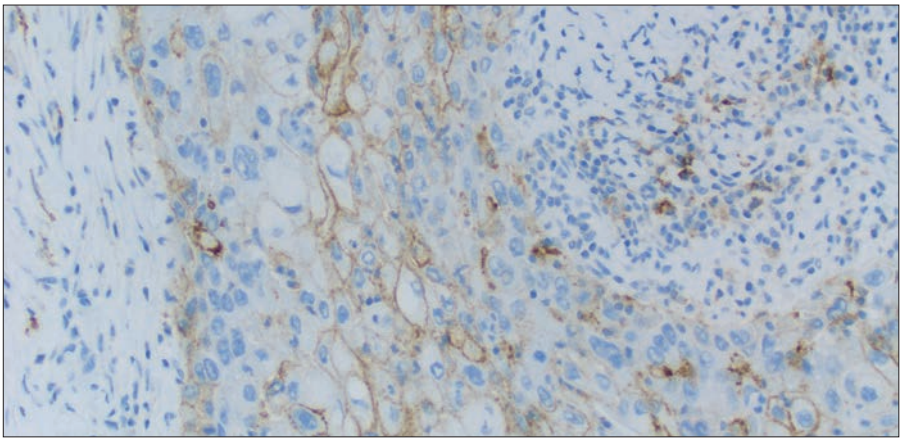


Figure 51b: 20× magnification.

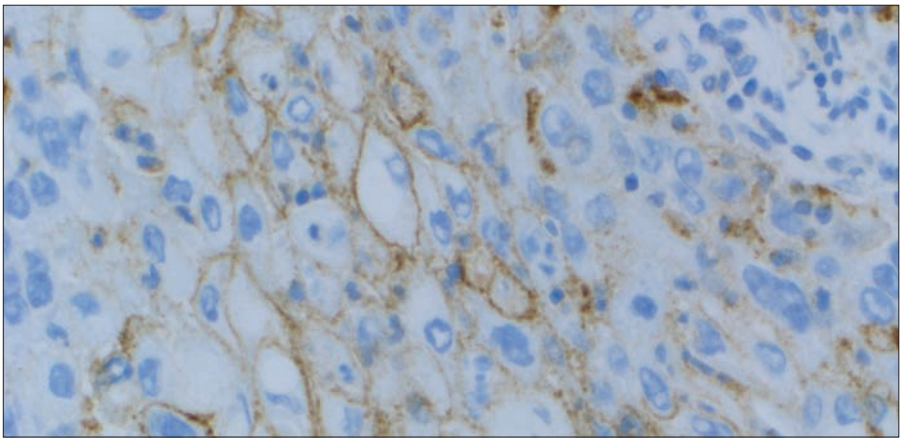


Figure 50c: 40× magnification.

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

Challenging Case 10: TPS 40–60%

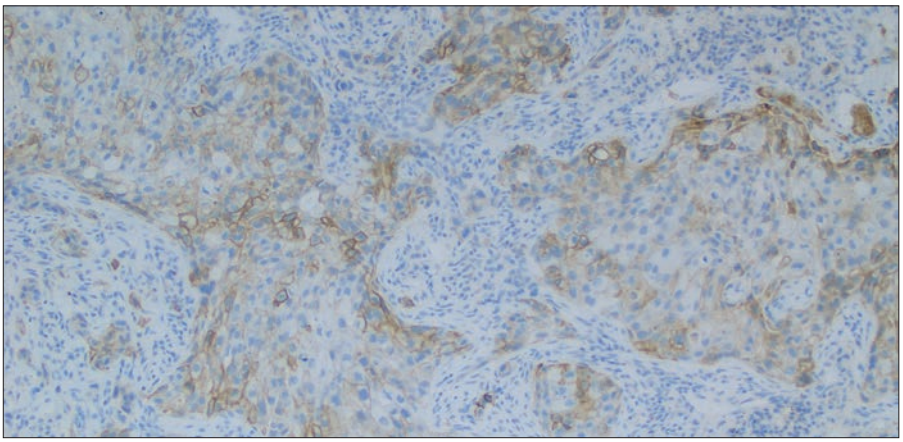


Figure 52a: 10× magnification.

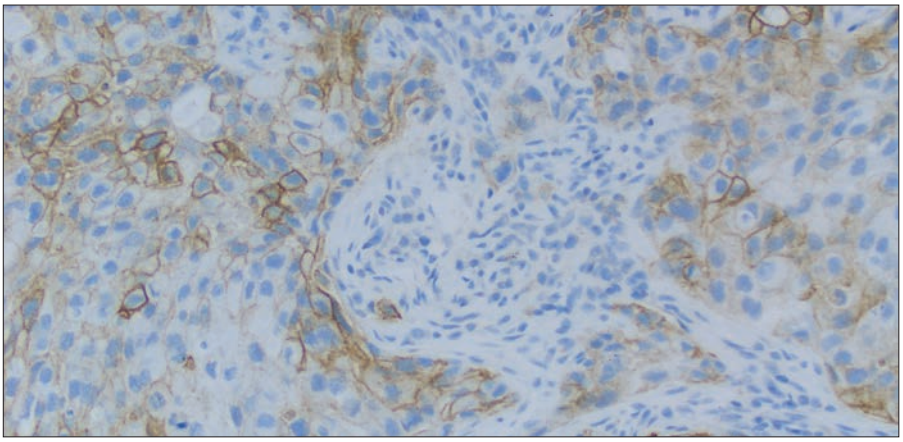


Figure 52b: 20× magnification.

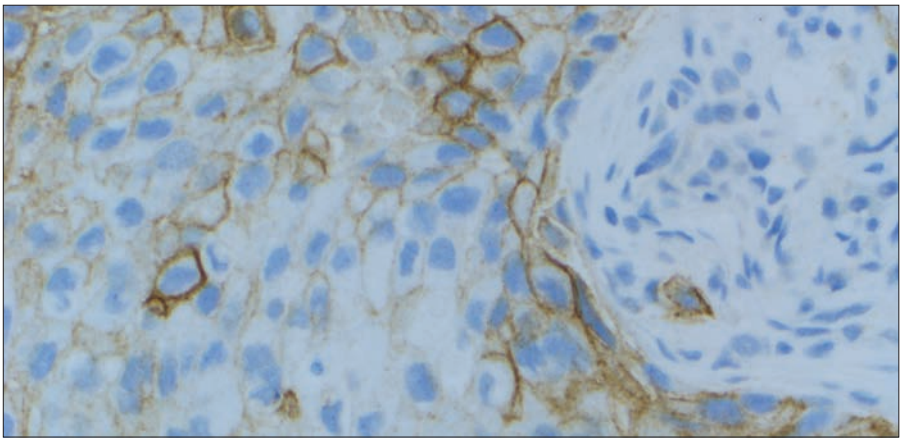


Figure 52c: 40× magnification.

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.

Non-specific Background Staining

Background staining is defined as diffuse, non-specific staining of a specimen. It is caused by several factors. These factors include, but are not limited to:

- Pre-analytic fixation and processing of the specimen
- Incomplete removal of paraffin from the section
- Incomplete rinsing of slides during staining
- Drying of slides; ensure slides remain wet with buffer while loading onto Autostainer Link 48 and prior to initiating run
- Improper deparaffinization procedure
- Incomplete rinsing of reagents from slides

The non-specific background staining of the NCR-stained test specimen is useful in determining the level of background staining in the positive test specimen. All specimens must have ≤ 1+ non-specific background staining.

The use of fixatives other than neutral buffered formalin may be a source of background staining and is not recommended. Background staining with PD-L1 IHC 22C3 pharmDx is rare.

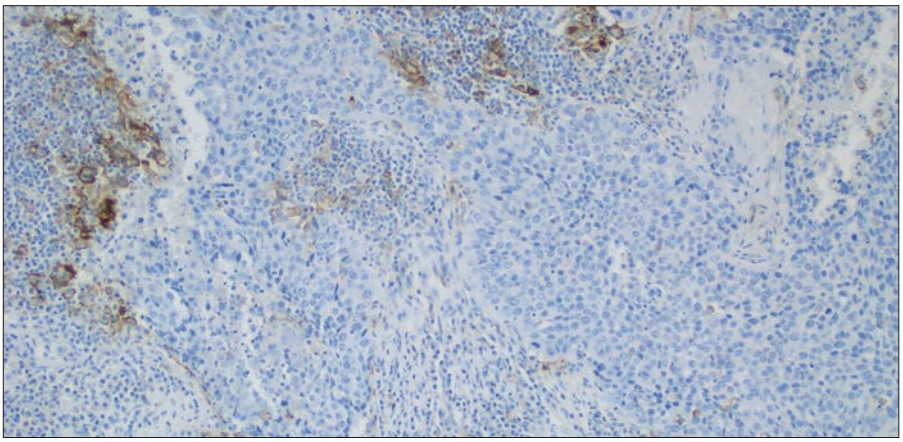


Figure 53: NSCLC specimen stained with PD-L1 primary antibody exhibiting acceptable non-specific background staining (20× magnification).

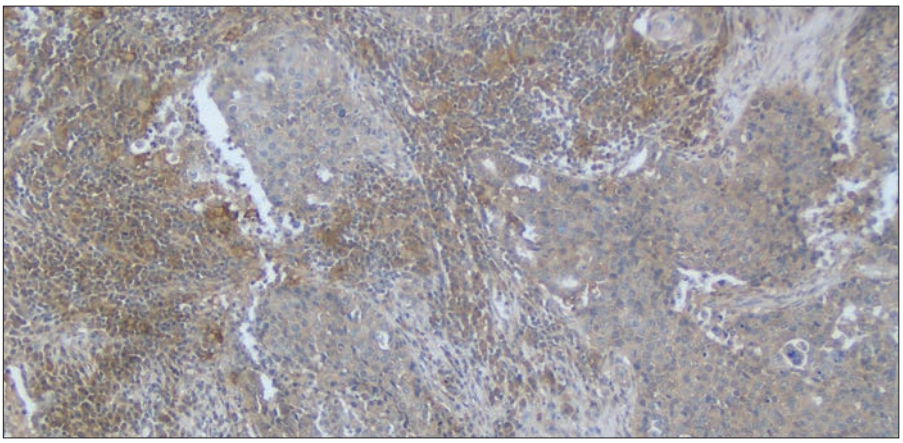


Figure 54: NSCLC specimen stained with PD-L1 primary antibody exhibiting unacceptable non-specific background staining (> 1+) (20× magnification).

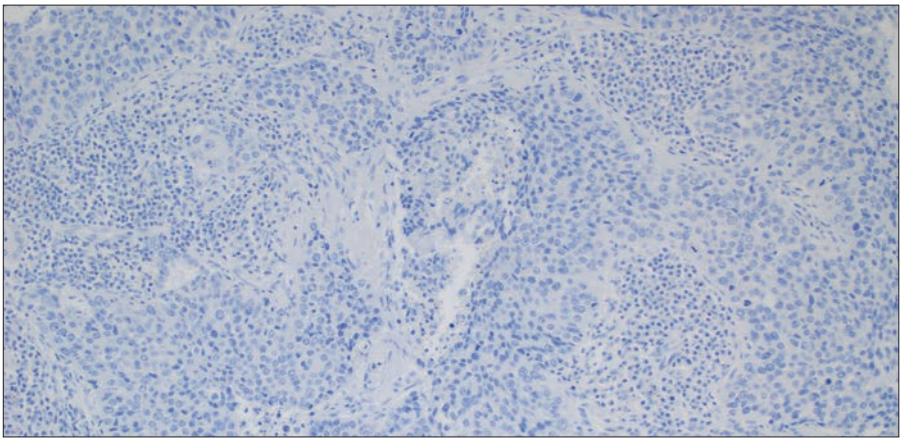


Figure 55: NSCLC specimen stained with NCR exhibiting acceptable non-specific background staining (20× magnification).

Key Point

All specimens must have ≤ 1+ non-specific background 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 non-staining in the central portion. Only PD-L1 staining at the edge of the tissue section is excluded from scoring.

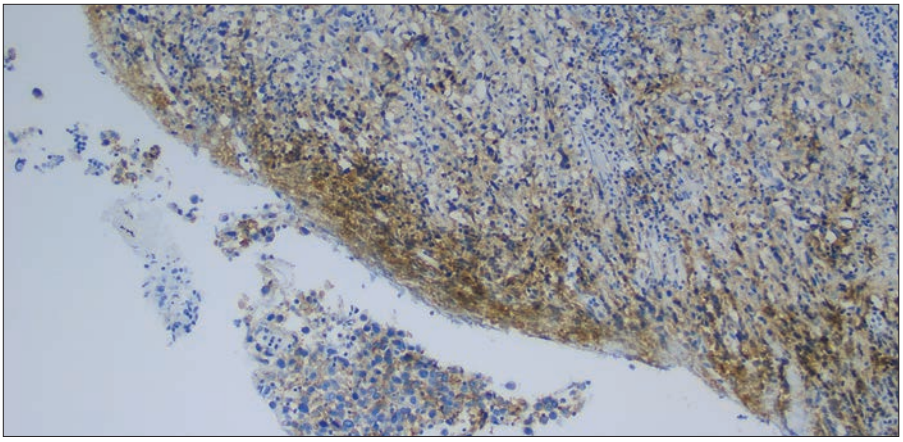


Figure 56a: NSCLC specimen stained with PD-L1 primary antibody exhibiting edge artifact staining; edge staining should be excluded from the scoring (4× magnification).

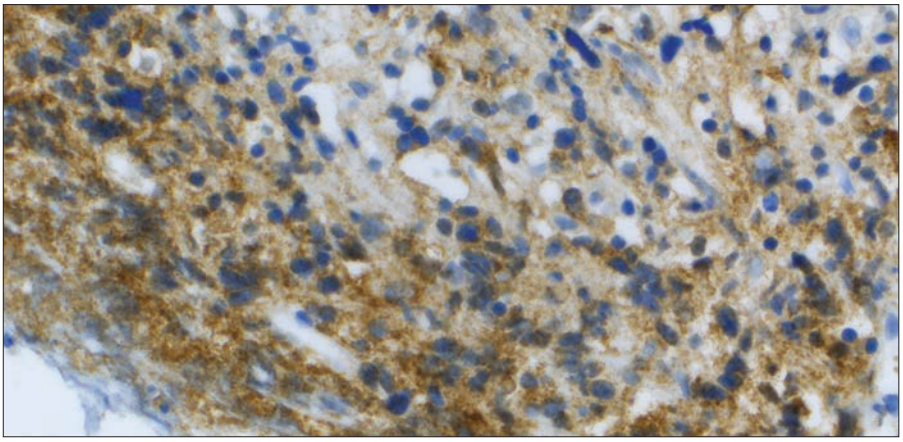


Figure 56b: NSCLC specimen stained with PD-L1 primary antibody exhibiting edge artifact staining; edge staining should be excluded from the scoring (20× magnification).

Key Point

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

Crush Artifact

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

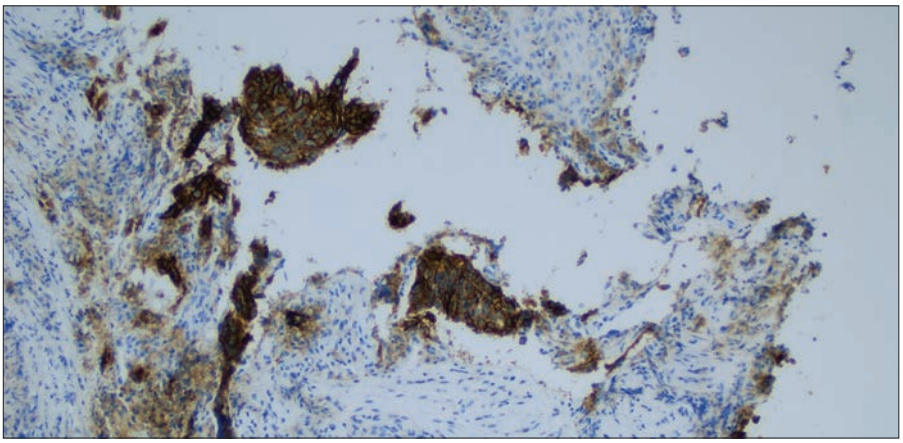


Figure 57: NSCLC specimen stained with PD-L1 primary antibody exhibiting crush artifact (10× magnification).

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.

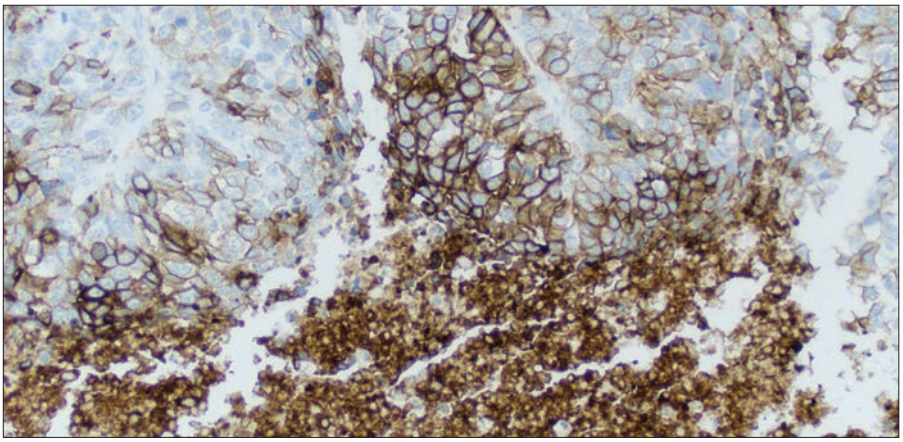


Figure 58: NSCLC specimen stained with PD-L1 primary antibody exhibiting strong staining of necrosis and viable tumor cells; necrosis staining should be excluded from the scoring (20× magnification).

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.

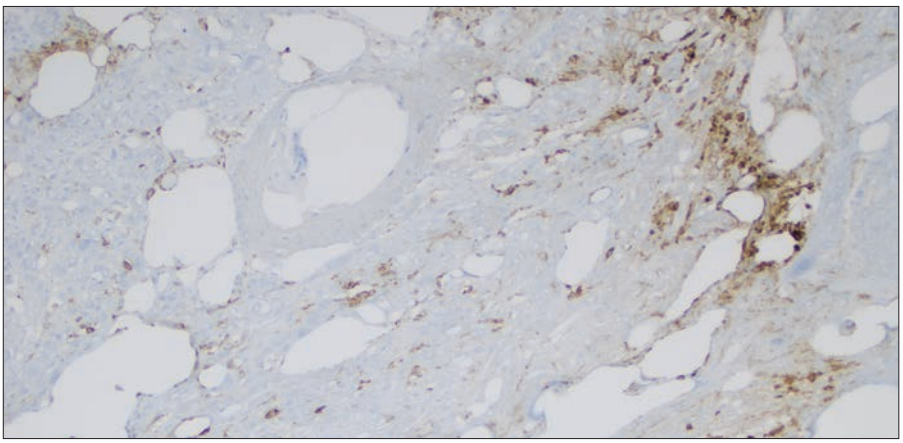


Figure 59: NSCLC specimen stained with PD-L1 primary antibody exhibiting poor tissue fixation (10× magnification).

Key Point

Proper fixation is important for accurate diagnosis

Control Cell Line (CCL) Appendix

Passing CCL

Passing PD-L1 Negative CCL

- No specific staining
- Non-specific staining is < 1+ intensity
- The presence of ≤ 10 total cells with distinct plasma membrane staining, or cytoplasmic staining with ≥ 1+ intensity within the boundaries of the MCF-7 cell pellet are acceptable

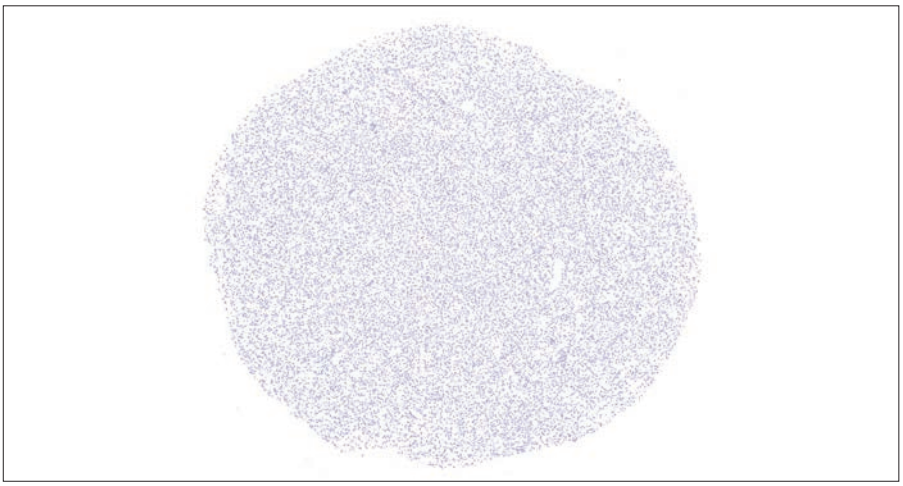


Figure 60: MCF-7 cell pellet (2× magnification).

Passing PD-L1 Positive CCL

- Cell membrane staining of ≥ 70% of cells
- ≥ 2+ average staining intensity
- Non-specific staining is < 1+ intensity

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

Borderline Passing CCL

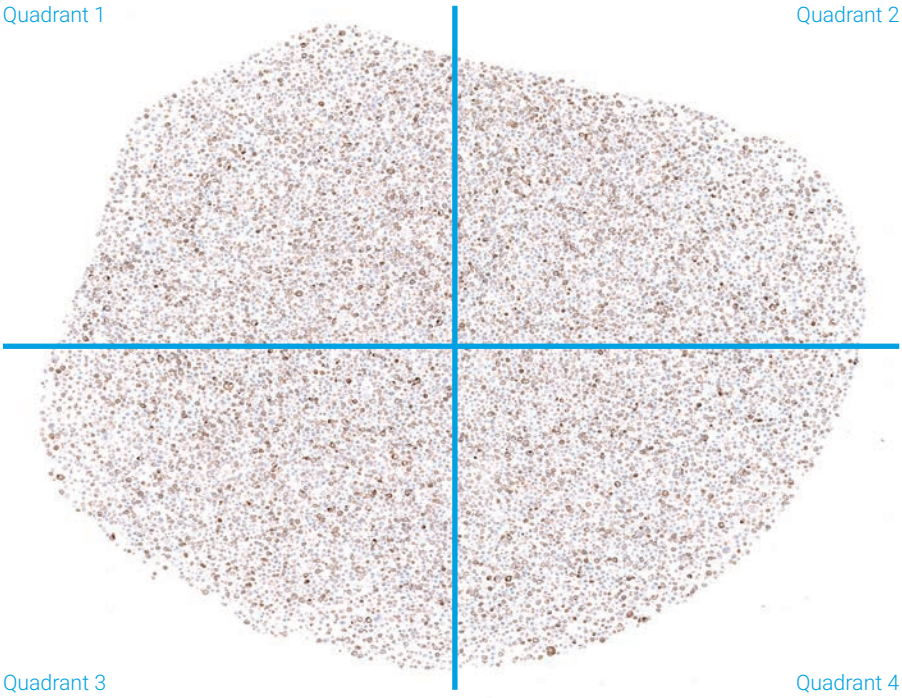
Borderline Passing vs. Passing PD-L1 Positive CCL

Borderline Passing PD-L1 positive CCL

Figure 62: 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 cells in the pellet, the cell pellet can be split into quadrants and inspected at 20× magnification.



Quadrant 1

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

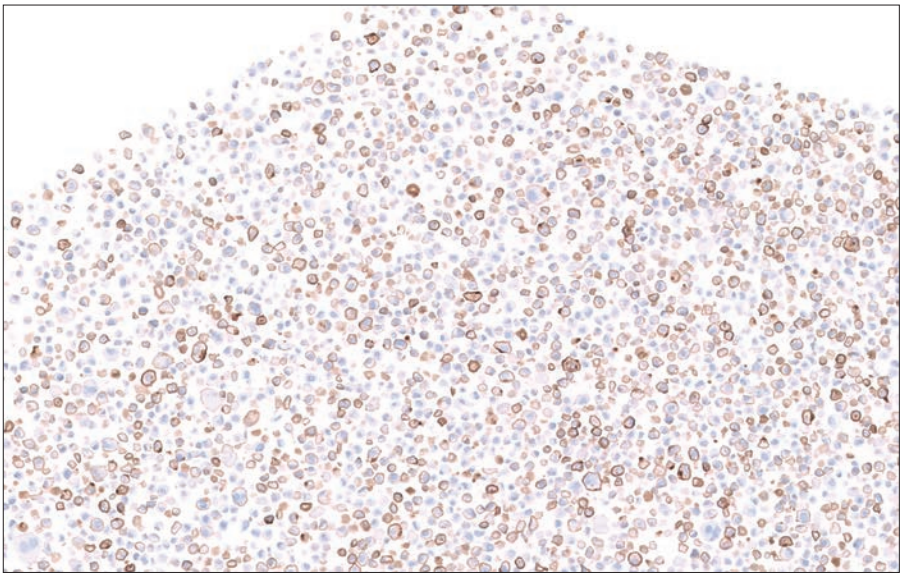


Figure 63: NCI-H226 cell pellet (5× magnification).

Quadrant 2

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

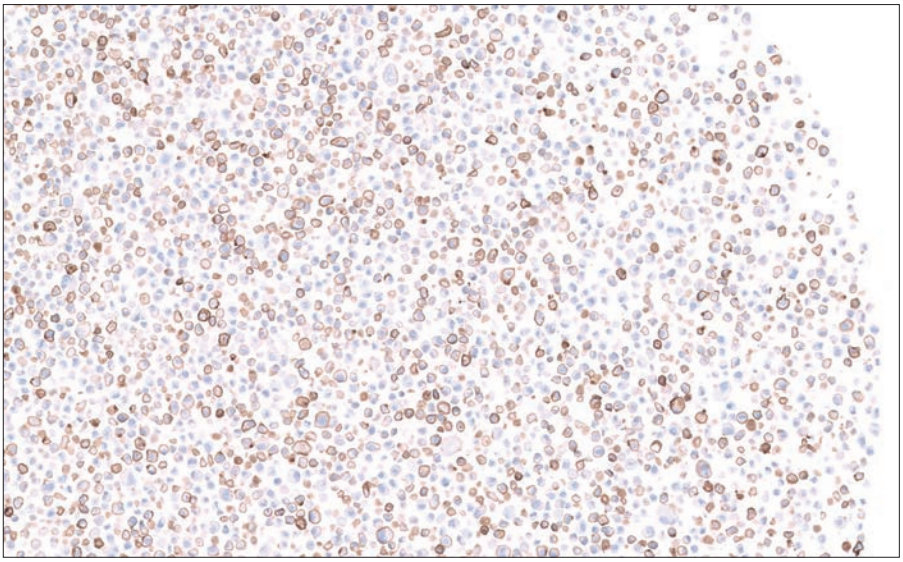


Figure 64: NCI-H226 cell pellet (5× magnification).

Quadrant 3

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

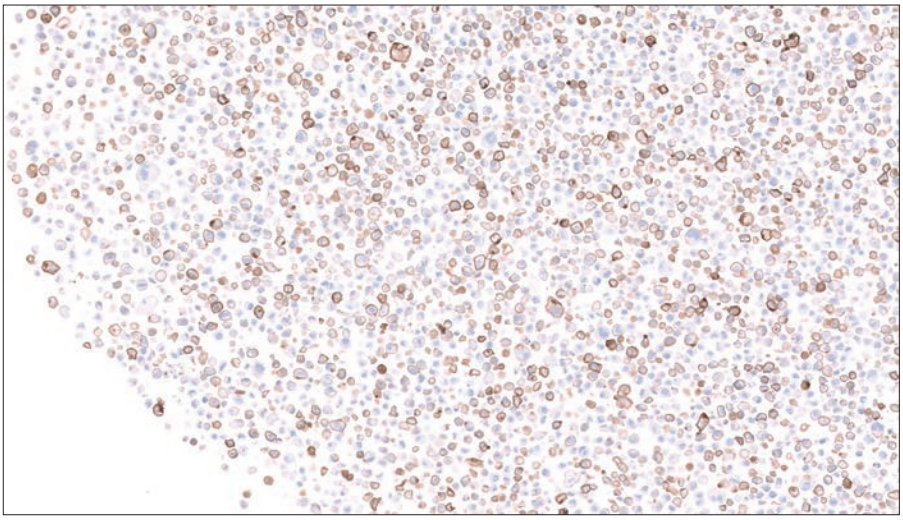


Figure 65: NCI-H226 cell pellet (5× magnification).

Quadrant 4

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

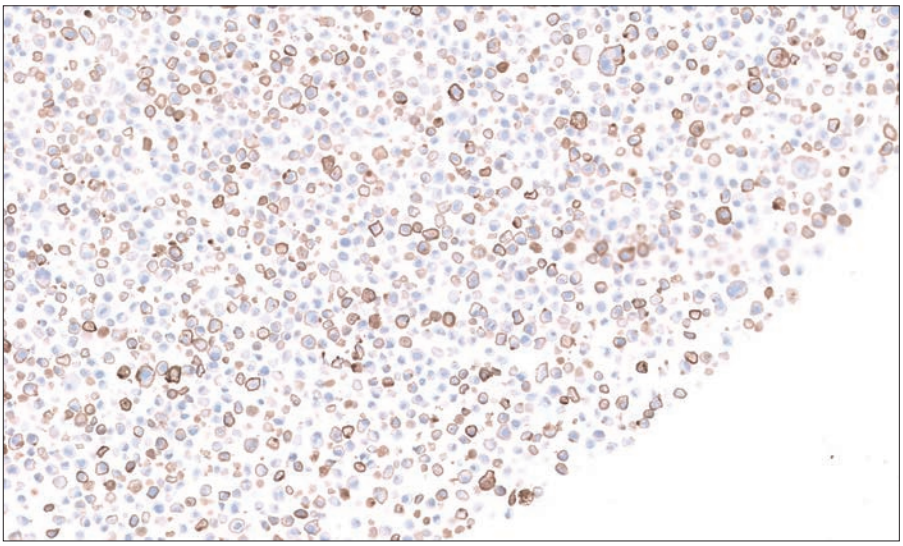
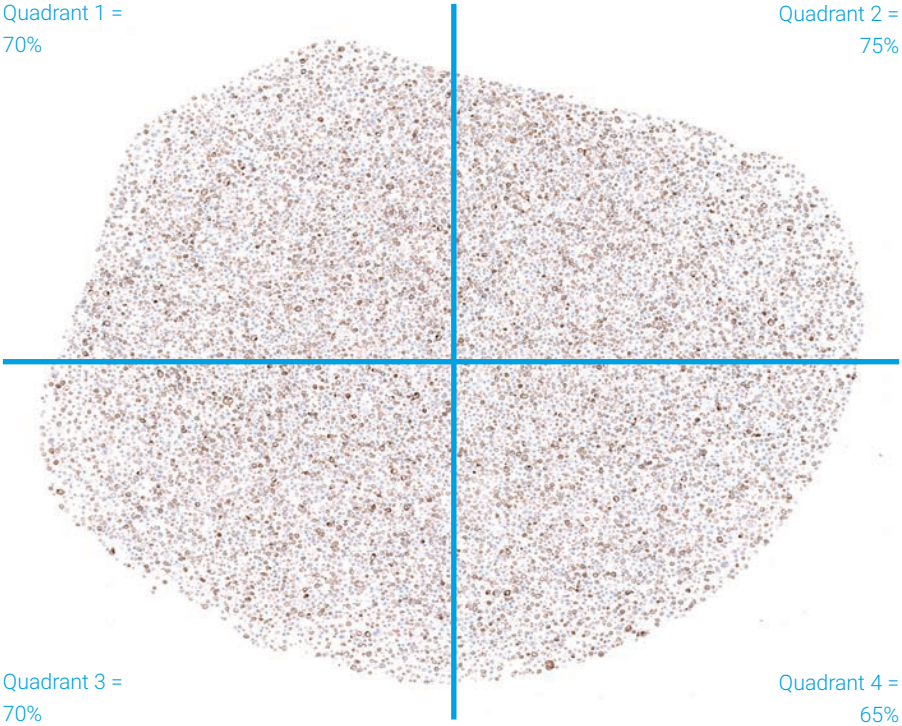


Figure 66: NCI-H226 cell pellet (5× magnification).

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 in the pellet is ≥ 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 in the cell pellet is ≥ 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 specific staining
- Non-specific staining is < 1+ intensity
- The presence of ≤ 10 total cells with distinct plasma membrane staining, or cytoplasmic staining with ≥ 1+ intensity within the boundaries of the PD-L1 negative cell pellet are acceptable

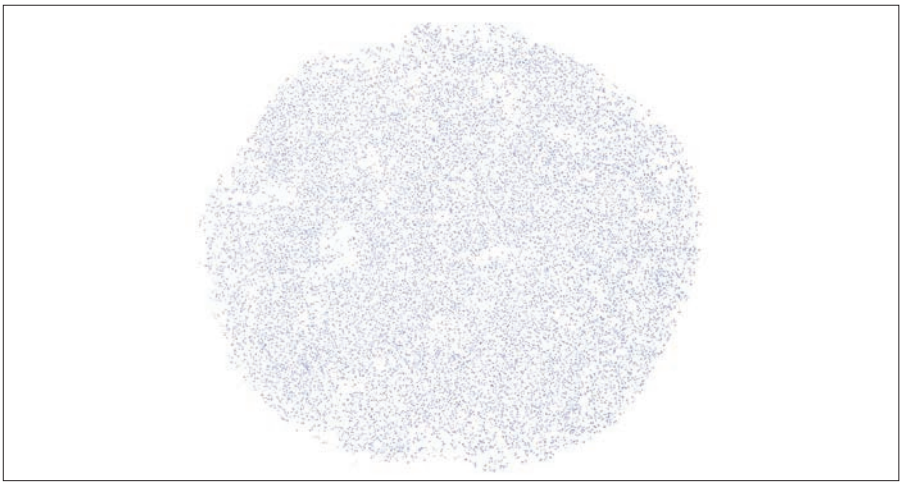


Figure 67: MCF-7 cell pellet (2× magnification).

Failed PD-L1 positive CCL

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

Figure 68: NCI-H226 cell pellet (2× magnification).

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

Failed PD-L1 positive CCL (10×)

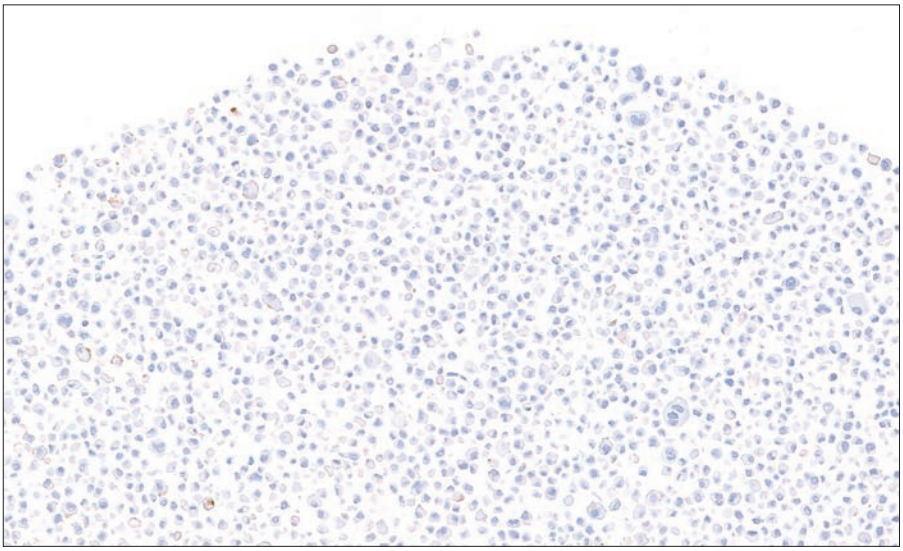


Figure 69: NCI-H226 cell pellet (10× magnification).

Failed PD-L1 positive CCL (20×)

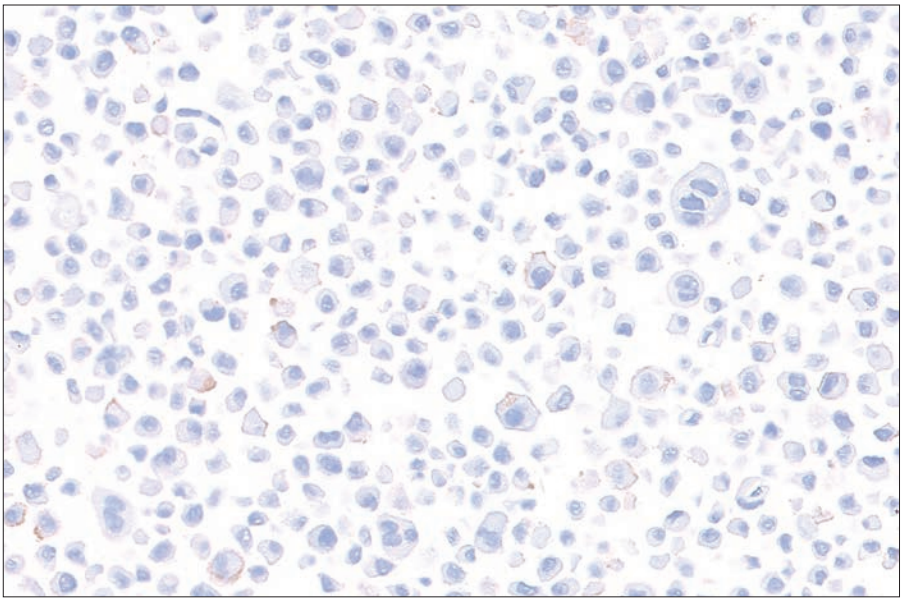


Figure 70: NCI-H226 cell pellet (20× magnification).

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

Passing PD-L1 negative CCL

- No specific staining
- Non-specific staining is < 1+ intensity
- The presence of ≤ 10 total cells with distinct plasma membrane staining, or cytoplasmic staining with ≥ 1+ intensity within the boundaries of the PD-L1 negative cell pellet are acceptable

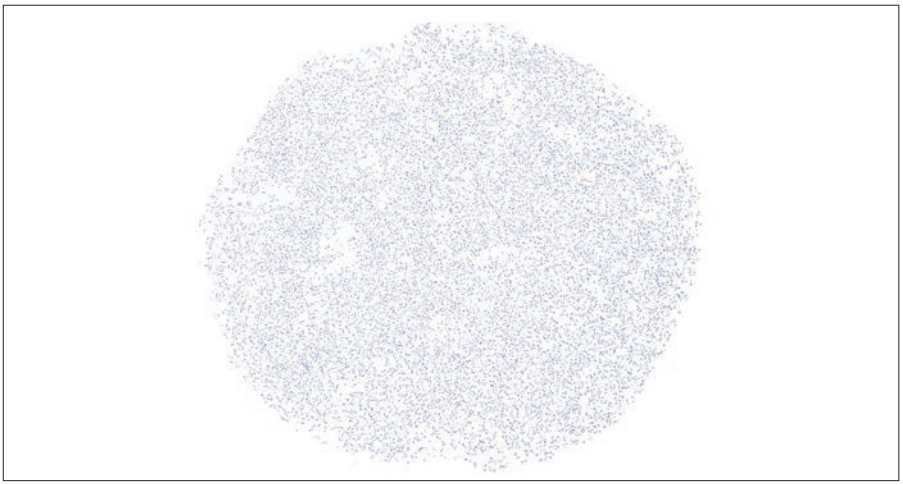


Figure 71: MCF-7 cell pellet (2× magnification).

Failed PD-L1 positive CCL

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

Figure 72: NCI-H226 cell pellet (2× magnification).

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

Failed PD-L1 positive CCL (10×)

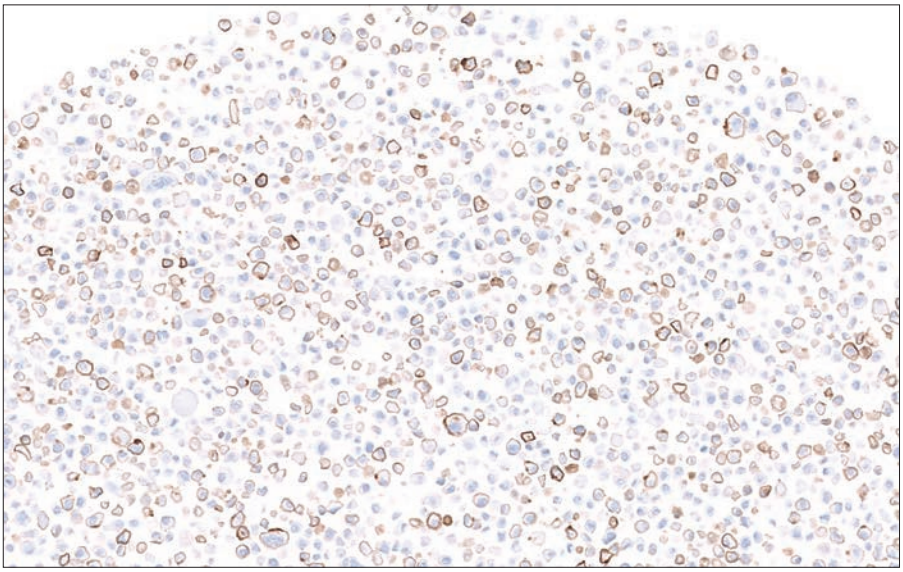


Figure 73: NCI-H226 cell pellet (10× magnification).

Failed PD-L1 positive CCL (20×)

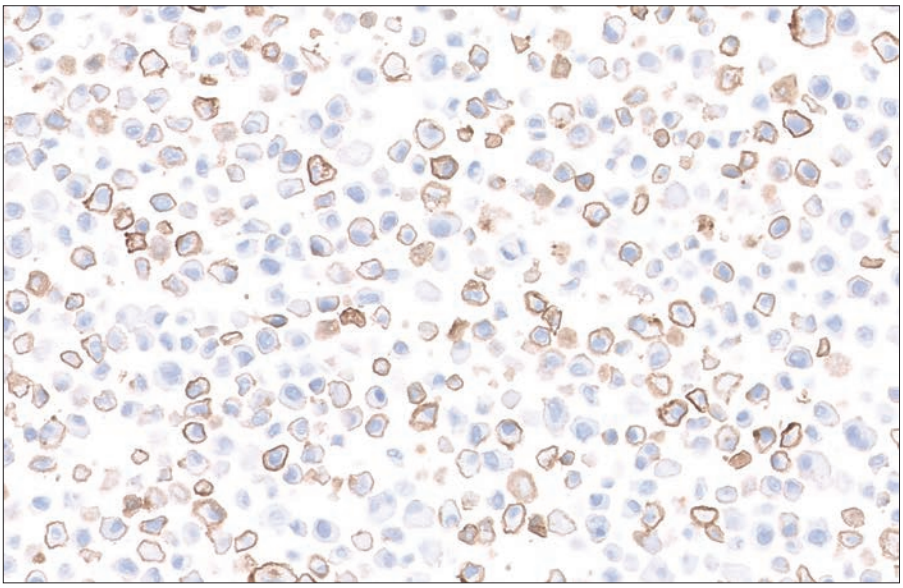


Figure 74: NCI-H226 cell pellet (20× magnification).

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

Passing PD-L1 positive CCL

- Cell membrane staining of ≥ 70% of cells
- ≥ 2+ average staining intensity
- Non-specific staining is < 1+

Figure 75: NCI-H226 cell pellet (2× magnification).

Failed PD-L1 negative CCL

- Non-specific (nuclear) staining is ≥ 1+ staining intensity
- There are > 10 total cells with distinct plasma membrane or cytoplasmic staining that is ≥ 1+ intensity

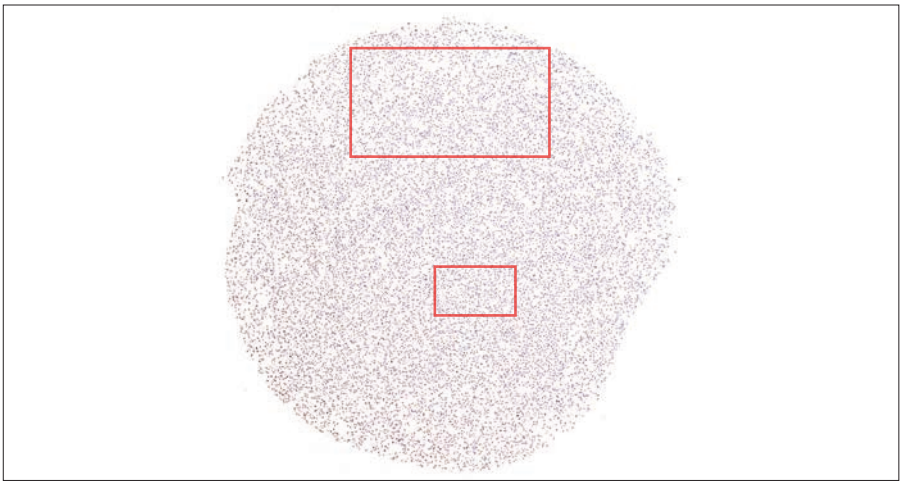


Figure 76: MCF-7 cell pellet (2× magnification).

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

Failed PD-L1 negative CCL (10×)

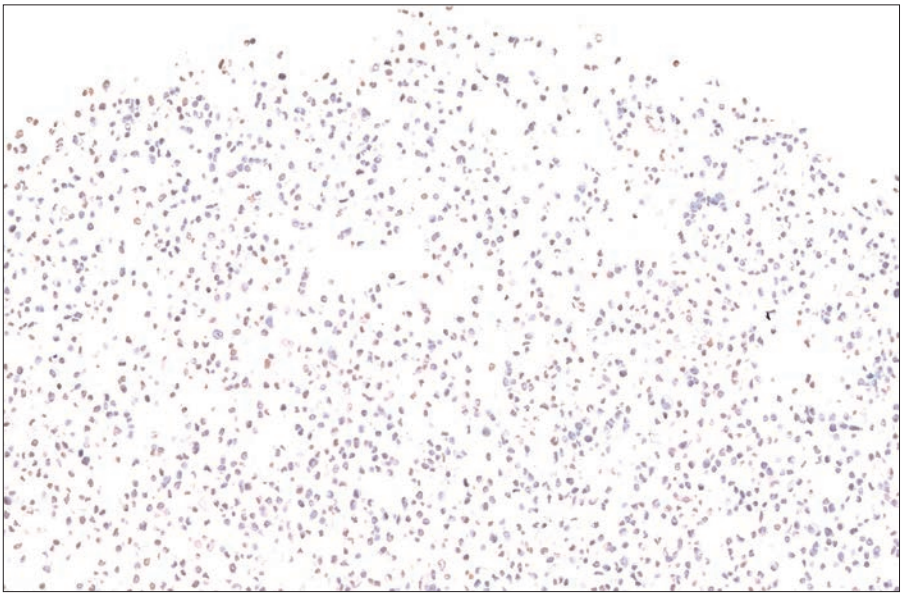


Figure 77: MCF-7 cell pellet (10× magnification).

Failed PD-L1 negative CCL (20×)

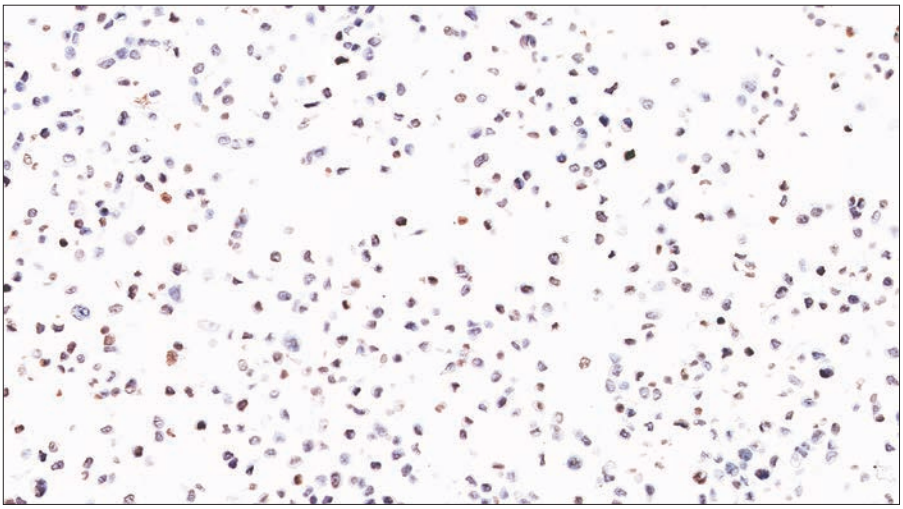


Figure 78: MCF-7 cell pellet (20× magnification).

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 EnVision FLEX Wash Buffer (20×) (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 EnVision FLEX Wash Buffer (20×) (Code K8007)
Weak staining of specimen slides or of the positive cell line on the Agilent-provided Control Slide	Inadequate target retrieval	Verify that the 3-in-1 pre-treatment procedure was correctly performed
	Inappropriate wash buffer used	Use only EnVision FLEX Wash Buffer (20×) (Code K8007)
Excessive background staining of slides	Paraffin incompletely removed	Verify that the 3-in-1 pre-treatment procedure was correctly performed
	Slides dried while loading onto Autostainer Link 48	Ensure slides remain wet with buffer while loading and prior to initiating run
	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
Tissue detached from slides	Use of incorrect microscope slides	Use Dako FLEX IHC Microscope Slides (Code K8020), or charged slides (such as Superfrost Plus)
	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	Only use EnVision FLEX Wash Buffer (20×) (Code K8007)

Continued on the next page.

For further troubleshooting help, contact your local Agilent representative.

Problem	Probable Cause	Suggested Action
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
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

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)

KEYNOTE-042: Controlled Trial of NSCLC Patients Naïve to Treatment

The safety and efficacy of pembrolizumab were also investigated in KEYNOTE-042, a multicenter, controlled study for the treatment of previously untreated locally advanced or metastatic NSCLC. The study design was similar to that of KEYNOTE-024 (see page 86), except that patients had PD-L1 expression with a TPS ≥ 1% based on PD-L1 IHC 22C3 pharmDx. Patients were randomized (1:1) to receive pembrolizumab at a dose of 200 mg every 3 weeks (n=637) or investigator's choice platinum-containing chemotherapy (n=637; including pemetrexed+carboplatin or paclitaxel+carboplatin; patients with non-squamous NSCLC could receive pemetrexed maintenance). Assessment of tumor status was performed every 9 weeks for the first 45 weeks, and every 12 weeks thereafter.

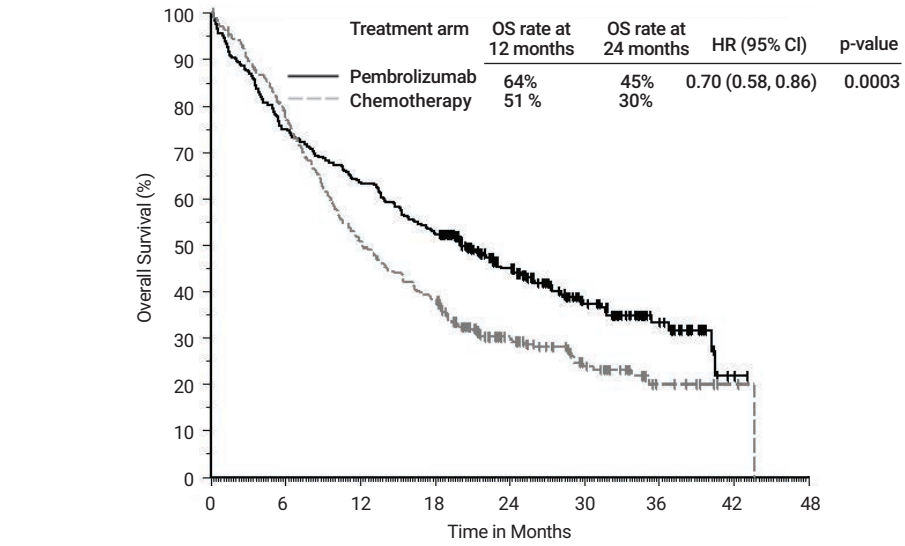
Among the 1,274 patients in KEYNOTE-042, 599 (47%) had tumors that expressed PD-L1 with TPS ≥ 50% based on PD-L1 IHC 22C3 pharmDx. The baseline characteristics of these 599 patients included: median age 63 years (45% age 65 or older); 69% male; 63% White and 32% Asian; 17% Hispanic or Latino; and ECOG performance status 0 and 1 in 31% and 69%, respectively. Disease characteristics were squamous (37%) and non-squamous (63%); stage IIIA (0.8%); stage IIIB (9%); stage IV (90%); and treated brain metastases (6%).

The primary efficacy outcome measure was OS. Secondary efficacy outcome measures were PFS and ORR (as assessed by BICR using RECIST 1.1). The trial demonstrated a statistically significant improvement in OS for patients whose tumors expressed PD-L1 TPS ≥ 1% randomized to pembrolizumab monotherapy compared to chemotherapy (HR 0.82; 95% CI 0.71, 0.93 at the final analysis) and in patients whose tumors expressed PD-L1 TPS ≥ 50% randomized to pembrolizumab monotherapy compared to chemotherapy. Table 6 summarizes key efficacy measures for the TPS ≥ 50% population at the final analysis performed at a median follow-up of 15.4 months. The Kaplan-Meier curve for OS for the TPS ≥ 50% population based on the final analysis is shown in Figure 79.

Table 6: Efficacy Results (PD-L1 TPS ≥ 50%) in KEYNOTE-042

Endpoint	Pembrolizumab 200 mg every 3 weeks n=299	Chemotherapy n=300
OS		
Number (%) of patients with event	180 (60%)	220 (73%)
Hazard ratio* (95% CI)	0.70 (0.58, 0.86)	
p-Value †	0.0003	
Median in months (95% CI)	20.0 (15.9, 24.2)	12.2 (10.4, 14.6)
PFS		
Number (%) of patients with event	238 (80%)	250 (83%)
Hazard ratio* (95% CI)	0.84 (0.70, 1.01)	
Median in months (95% CI)	6.5 (5.9, 8.5)	6.4 (6.2, 7.2)
Objective Response Rate		
ORR % (95% CI)	39% (34, 45)	32% (27, 38)
Complete response %	1%	0.3%
Partial response %	38%	32%
Response Duration‡		
Median in months (range)	22.0 (2.1+, 36.5+)	10.8 (1.8+, 30.4+)
% with duration ≥ 18 months	57%	34%

** Hazard ratio (pembrolizumab compared to chemotherapy) based on the stratified Cox proportional hazard model*
† Based on stratified log-rank test
‡ Based on patients with a best objective response as confirmed complete or partial response



Number at Risk

Pembrolizumab:	299	224	190	157	94	50	21	1	0
Chemotherapy:	300	231	151	113	59	31	8	2	0

Figure 79: Kaplan-Meier curve for overall survival by treatment arm in KEYNOTE-042 (patients with PD-L1 expression TPS ≥ 50%, intent to treat population).

The results of a post-hoc exploratory subgroup analysis indicated a trend towards reduced survival benefit of pembrolizumab compared to chemotherapy, during both the first 4 months and throughout the entire duration of treatment, in patients who were never-smokers. However, due to the exploratory nature of this subgroup analysis, no definitive conclusions can be drawn.

KEYNOTE-024: Controlled Study of NSCLC Patients Naïve to Treatment

The safety and efficacy of pembrolizumab were investigated in KEYNOTE-024, a multicenter, open label, controlled study for the treatment of previously untreated metastatic NSCLC. Patients had PD-L1 expression with a TPS ≥ 50% based on PD-L1 IHC 22C3 pharmDx. Patients were randomized (1:1) to receive pembrolizumab at a dose of 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; patients with non-squamous NSCLC could receive pemetrexed maintenance). Patients were treated with pembrolizumab until unacceptable toxicity or disease progression. Treatment could continue beyond disease progression if the patient was clinically stable and was considered to be deriving clinical benefit by the investigator. Patients without disease progression could be treated for up to 24 months. The study excluded 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. Assessment of tumor status was performed every 9 weeks. Patients on chemotherapy who experienced independently-verified progression of disease were able to crossover and receive pembrolizumab.

Among the 305 patients in KEYNOTE-024, baseline characteristics were: median age 65 years (54% age 65 or older); 61% male; 82% White, 15% Asian; and ECOG performance status 0 and 1 in 35% and 65%, respectively. Disease characteristics were squamous (18%) and non-squamous (82%); M1 (99%); and brain metastases (9%).

The primary efficacy outcome measure was PFS as assessed by blinded independent central review (BICR) using RECIST 1.1. Secondary efficacy outcome measures were OS and ORR (as assessed by BICR using RECIST 1.1). Table 7 summarizes key efficacy measures for the entire intent to treat (ITT) population. PFS and ORR results are reported from an interim analysis at a median follow up of 11 months. OS results are reported from the final analysis at a median follow up of 25 months.

Table 7: Efficacy Results in KEYNOTE-024

Endpoint	Pembrolizumab 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	73 (47%)	96 (64%)
Hazard ratio* (95% CI)	0.63 (0.47, 0.86)	
p-Value †	0.002	
Median in months (95% CI)	30.0 (18.3, NA)	14.2 (9.8, 19.0)
Objective Response Rate		
ORR % (95% CI)	45% (37, 53)	28% (21, 36)
Complete response %	4%	1%
Partial response %	41%	27%
Response Duration‡		
Median in months (range)	Not reached (1.9+, 14.5+)	6.3 (2.1+, 12.6+)
% with duration ≥ 6 months	88%§	59%¶

** Hazard ratio (pembrolizumab compared to chemotherapy) based on the stratified Cox proportional hazard model*
† Based on stratified log-rank test
‡ Based on patients with a best objective response as confirmed complete or partial response
§ Based on Kaplan-Meier estimates; includes 43 patients with responses of 6 months or longer
¶ Based on Kaplan-Meier estimates; includes 16 patients with responses of 6 months or longer
NA = not available

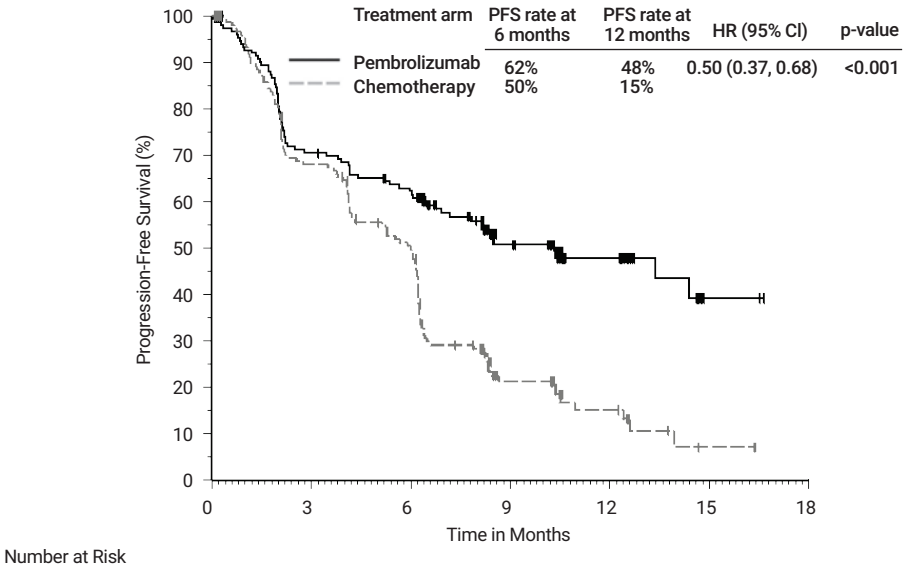


Figure 80: Kaplan-Meier curve for progression-free survival by treatment arm in KEYNOTE-024 (intent to treat population).

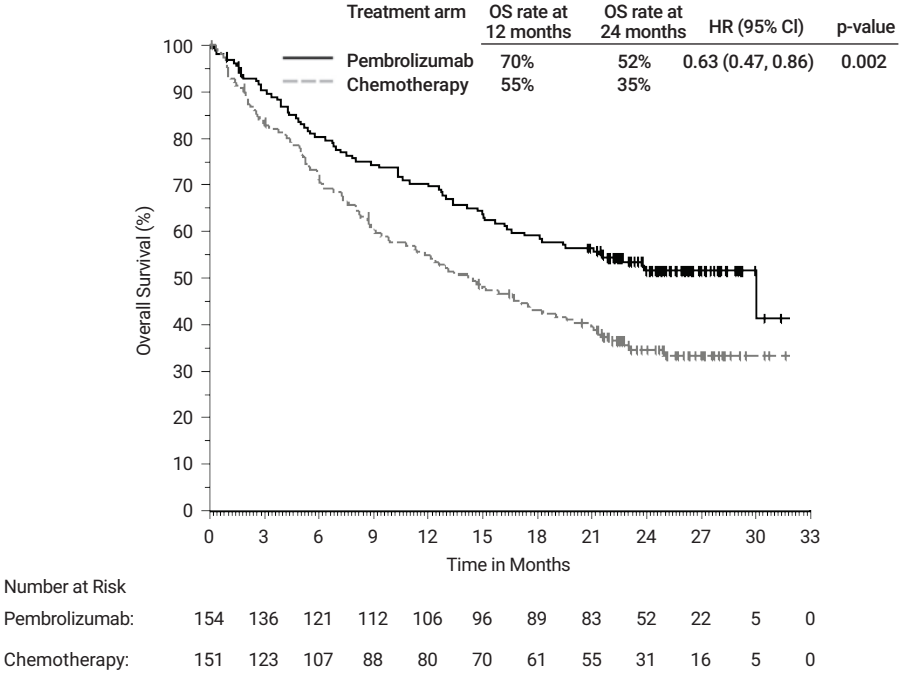


Figure 81: Kaplan-Meier curve for overall survival by treatment arm in KEYNOTE-024 (intent to treat population).

In a subgroup analysis, a reduced survival benefit of pembrolizumab compared to chemotherapy was observed in the small number of patients who were never-smokers; however, due to the small number of patients, no definitive conclusions can be drawn from these data.

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

The clinical benefit of PD-L1 IHC 22C3 pharmDx was investigated in KEYNOTE-010, a multicenter, open-label, randomized clinical study conducted to assess the safety and efficacy of KEYTRUDA in patients with advanced NSCLC previously treated with platinum-containing chemotherapy. Patients had PD-L1 expression with a TPS ≥ 1% based on a clinical trial assay version of PD-L1 IHC 22C3 pharmDx (CTA). Patients with EGFR activation mutation or ALK translocation also had disease progression on approved therapy for these mutations prior to receiving pembrolizumab. Patients were randomized (1:1:1) to receive pembrolizumab at a dose of 2 (n=344) or 10 mg/kg (n=346) every 3 weeks or docetaxel at a dose of 75 mg/m^2 every 3 weeks (n=343) until disease progression or unacceptable toxicity. The trial excluded 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. 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 US 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 TPS ≥ 1% and samples from 94 patients did not have PD-L1 expression (TPS < 1%). In these 413 patients with PD-L1 expression TPS ≥ 1%, 163 patient specimens had PD-L1 expression TPS ≥ 50%.

The level of agreement achieved between the CTA and PD-L1 IHC 22C3 pharmDx is shown in Table 8.

Table 8: CTA vs. PD-L1 IHC 22C3 pharmDx Agreement

Agreement Rates	PD-L1 Cutoff	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 TPS ≥ 1% 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 9 and 10. KEYTRUDA demonstrated durable clinical benefit in NSCLC patients with PD-L1 expression TPS ≥ 1%, which was enhanced in patients with 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 subpopulation with PD-L1 expression TPS ≥ 50% for the overall clinical study (PD-L1 expression TPS ≥ 1% based on CTA) and in the population with PD-L1 expression determined 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 82. Efficacy results were similar for the 2 mg/kg and 10 mg/kg KEYTRUDA arms.

Table 9: Response to KEYTRUDA in Previously Treated NSCLC Patients: Overall Clinical Study and Patients with PD-L1 Expression TPS ≥ 1% as Determined by PD-L1 IHC 22C3 pharmDx

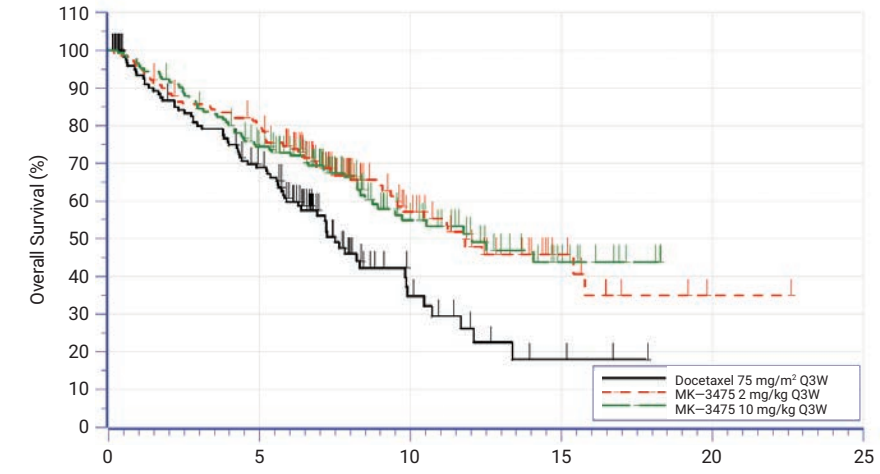
Endpoint	KEYTRUDA 2 mg/kg every 3 weeks		KEYTRUDA 10 mg/kg every 3 weeks		Docetaxel 75 mg/m^2 every 3 weeks	
	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx
Number of patients	344	140	346	142	343	131
OS						
Deaths (%)	172 (50%)	59 (42%)	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 (69%)	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 10: Response to KEYTRUDA in Previously Treated NSCLC Patients: Overall Clinical Study and Patients with PD-L1 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



n at risk					
Docetaxel 75 mg/m² Q3W					
131	78	14	3	0	0
MK—3475 2 mg/kg Q3W					
140	109	36	10	1	0
MK—3475 10 mg/kg Q3W					
142	99	36	10	0	0

Figure 82: 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)

Regeneron Study 1624: First-line Treatment of Locally Advanced NSCLC In Patients Who Are Not Candidates for Definitive Chemoradiation, or Metastatic NSCLC

The efficacy and safety of LIBTAYO compared with platinum-doublet chemotherapy in patients with locally advanced NSCLC who were not candidates for definitive chemoradiation, or with metastatic NSCLC who had tumor PD-L1 expression of TPS ≥ 50% using PD-L1 IHC 22C3 pharmDx were evaluated in Study 1624, a randomized, open-label, multi-center trial.

The trial was designed to enroll patients with tumor PD-L1 expression of TPS ≥ 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 ≥ 50% using PD-L1 IHC 22C3 pharmDx according to the product labeling.

The study excluded patients with EGFR, ALK or ROS1 genomic tumor aberrations, ECOG performance score (PS) ≥ 2, medical conditions that required systemic immunosuppression, uncontrolled infection with hepatitis B (HBV) or hepatitis C (HCV) or human immunodeficiency virus (HIV), history of interstitial lung disease, who were never smokers or who had an autoimmune disease that required systemic therapy within 2 years of treatment. Treatment of brain metastases was permitted, and patients could be enrolled if they had been adequately treated and had neurologically returned to baseline for at least 2 weeks prior to randomization. Radiological confirmation of stability or response was not required.

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 independent review committee (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 ≥ 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 objective response rate (ORR).

The study population characteristics of patients with PD-L1 expression of TPS ≥ 50% are included in Table 11.

Table 11: Summary of Baseline Patient and Disease Characteristics in the Population with TPS ≥ 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 ≥ 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 ≥ 50%.

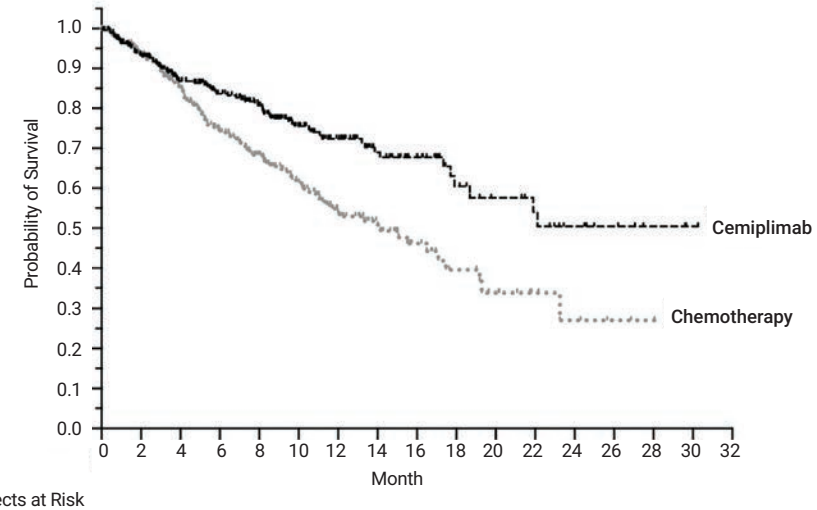
In the population with TPS ≥ 50%, the trial demonstrated statistically significant improvement in OS and PFS for patients randomized to LIBTAYO as compared with chemotherapy. Results were similar to the efficacy results for the ITT population.

Efficacy results for the population with PD-L1 expression of TPS ≥ 50% are presented in Table 12 and in Figures 83 and 84.

Table 12: Efficacy Results from Study 1624 in Non-small Cell Lung Cancer in the Population with TPS ≥ 50%

	TPS ≥ 50% Population (N=563)	
Endpoints[a]	**LIBTAYO 350 mg every 3 weeks n=283**	**Chemotherapy n=280**
Overall Survival (OS)		
Number of deaths (%)	70 (24.7)	105 (37.5)
Median in months (95% CI)[b]	NR (17.9, NE)	14.2 (11.2, 17.5)
Hazard ratio (95% CI)[c]	0.57 (0.42, 0.77)	
p-Value[d]	0.0002	
OS rate at 12 months (95% CI)[b]	72.4 (65.6, 78.1)	53.9 (46.2, 61.1)
Progression-free Survival (PFS)		
Number of events (%)	147 (51.9)	197 (70.4)
Median in months (95% CI)[b]	8.2 (6.1, 8.8)	5.7 (4.5, 6.2)
Hazard ratio (95% CI)[c]	0.54 (0.43, 0.68)	
p-Value[d]	<0.0001	
PFS rate at 12 months (95% CI)[b]	40.7 (33.7, 47.5)	7.1 (3.6, 12.1)
Objective Response Rate (ORR) (%)[e,f]		
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 (DOR)[e]		
Median (months)[b]	16.7	6.0
Range (months)	1.9+, 23.3+	1.3+, 14.5+
Patients with observed DOR ≥ 6 months (%)	73 (65.8)	23 (40.4)

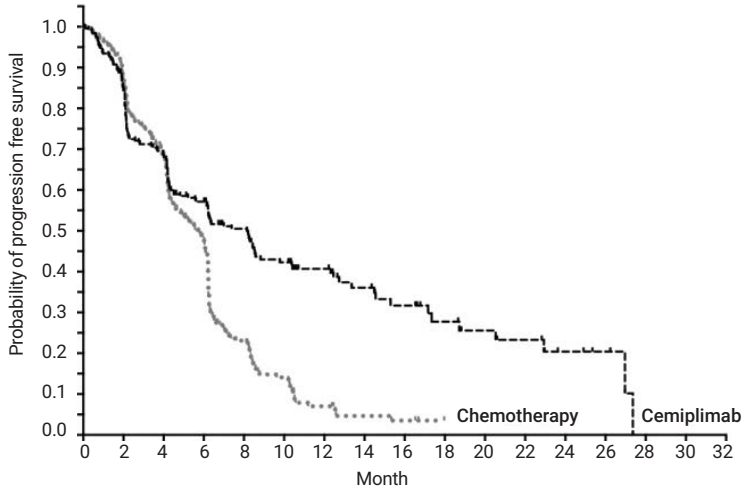
CI: Confidence interval; NE: Not evaluable; NR: Not reached
+: Ongoing response
[a] Median duration of follow-up: Cemiplimab: 10.8 months; Chemotherapy: 10.9 months
[b] Based on Kaplan-Meier estimates
[c] Based on stratified proportional hazards model
[d] Based on a two-sided p-value
[e] Not a pre-specified endpoint
[f] Based on 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 83: Kaplan-Meier curve for OS in the TPS ≥ 50% population.



Number of Subjects at Risk

Cemiplimab:	283	221	162	123	92	59	43	28	20	14	11	9	5	3	0	0	0
Chemotherapy:	280	220	157	104	42	28	8	4	3	0	0	0	0	0	0	0	0

Figure 84: Kaplan-Meier curve for PFS in the TPS ≥ 50% population.

References

- Keytruda [Summary of Product Characteristics]. European Medicines Agency; 2020.
- Libtayo [Summary of Product Characteristics]. European Medicines Agency; 2021.
- PD-L1 IHC 22C3 pharmDx [Instructions for Use]. Santa Clara, CA: Agilent Technologies, Inc.; 2021.
- Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1–positive non-small-cell lung cancer. *N Engl J Med.* 2016; 375(19):1823-1833.
- Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet.* 2016;387(10027):1540-1550.
- Sezer A, Kilickap S, Gümüş M, et al. Cemiplimab monotherapy for first-line treatment of advanced non-small-cell lung cancer with PD-L1 of at least 50%: a multicentre, open-label, global, phase 3, randomised, controlled trial. *Lancet.* 2021;397(10274):592-604.
- Roach C, Zhang N, Corigliano E, et al. Development of a companion diagnostic PD-L1 immunohistochemistry assay for pembrolizumab therapy in non-small-cell lung cancer. *Appl Immunohistochem Mol Morphol.* 2016;24:392-397.
- Dolled-Filhart M, Roach C, Toland G, et al. Development of a companion diagnostic for pembrolizumab in non-small cell lung cancer using immunohistochemistry for programmed death ligand-1. *Arch Pathol Lab Med.* 2016. doi:10.5858/arpa.2015-0542-OA.
- Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature.* 2014;515(7528):563-567.
- Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature.* 2014;515(7 528):568-571.
- Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* 2011;144(5):646-674.
- Sharpe AH, Freeman GJ. The B7-CD28 superfamily. *Nat Rev Immunol.* 2002;2(2):116-126.
- Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol.* 2008;26:677-704.
- Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med.* 2000;192(7):1027-1034.

- Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med.* 2002;8(8):793-800.
- Mu CY, Huang JA, Chen Y, Chen C, Zhang XG. High expression of PD-L1 in lung cancer may contribute to poor prognosis and tumor cells immune escape through suppressing tumor infiltrating dendritic cells maturation. *Med Oncol.* 2011;28(3):682-688.
- Gettinger S, Herbst RS. B7-H1/PD-1 blockade therapy in non-small cell lung cancer: current status and future direction. *Cancer J*. 2014;20(4):281-289.
- Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med.* 2012;366(26):2455-2465.
- Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med.* 2012;366(26):2443-2454.
- Boland JM, Kwon ED, Harrington SM, et al. Tumor B7-H1 and B7-H3 expression in squamous cell carcinoma of the lung. *Clin Lung Cancer*. 2013;14(2):157-163.
- Chen YB, Mu CY, Huang JA. Clinical significance of programmed death-1 ligand-1 expression in patients with non-small cell lung cancer: a 5-year-follow-up study. *Tumori*. 2012;98(6):751-755.
- Velcheti V, Schalper KA, Carvajal DE, et al. Programmed death ligand-1 expression in non-small cell lung cancer. *Lab Invest*. 2014;94(1):107-116.
- Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med*. 2015;372(21):2018-2028.

Notes

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 first clinical trial-proven companion diagnostic CE-IVD–marked as an aid in identifying patients with NSCLC for treatment with KEYTRUDA or LIBTAYO

Learn more:
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Europe
info_agilent@agilent.com

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

For countries outside of the European Union, see the local LIBTAYO product label for approved indications.

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