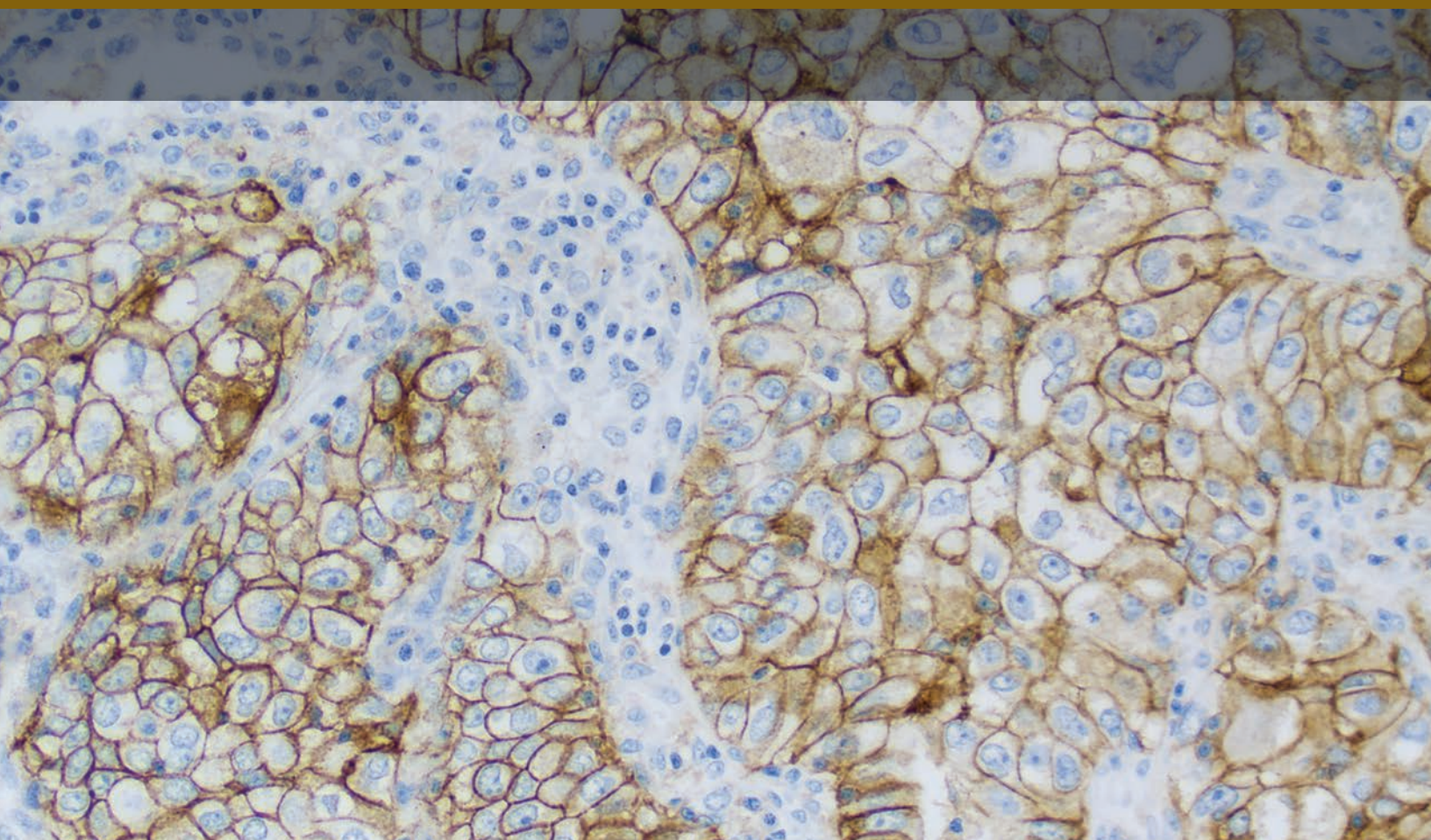


# PD-L1 IHC 22C3 pharmDx Interpretation Manual – NSCLC

For in vitro diagnostic use





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## 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) tissue using EnVision FLEX visualization system on Autostainer Link 48. PD-L1 protein expression 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. The specimen should be considered to have PD-L1 expression if  $TPS \geq 1\%$  and high PD-L1 expression if  $TPS \geq 50\%$ .

PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying NSCLC patients for treatment with KEYTRUDA® (pembrolizumab). See the KEYTRUDA product label for expression cutoff values guiding therapy in specific clinical circumstances.

# Introduction

PD-L1 IHC 22C3 pharmDx is the only clinical trial-proven companion diagnostic indicated as an aid in identifying patients with NSCLC for treatment with KEYTRUDA (pembrolizumab). 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. Expression of PD-L1 can help identify patients for anti-PD-1 immunotherapy.

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

PD-L1 IHC 22C3 pharmDx is considered a qualitative assay; however, diagnostic status using PD-L1 expression requires the determination of percentage of stained tumor cells.

Non-small cell lung cancer tissue specimens that are tested for PD-L1 expression are scored and divided into three levels based on a Tumor Proportion Score (TPS):

- TPS < 1%: No PD-L1 expression
- TPS 1–49%: PD-L1 expression
- TPS ≥ 50%: High PD-L1 expression

PD-L1 expression levels are used to inform patient eligibility for treatment with KEYTRUDA.

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 24.

## Photomicrographs

The included photomicrographs are of NSCLC unless otherwise noted.

## PD-L1 Overview

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

Programmed death-ligand 1 (PD-L1) is a trans-membrane 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.

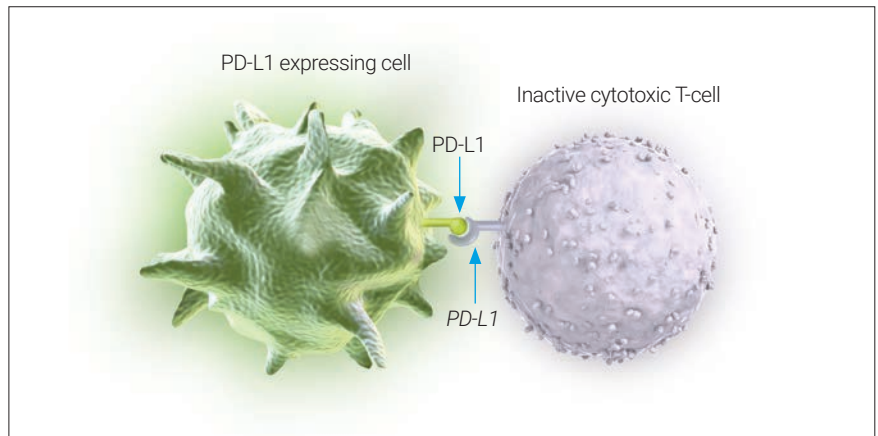
### Anti-PD-1 Therapy Enables the Immune Response Against Tumors

KEYTRUDA® (pembrolizumab) is an anti-PD-1 cancer immunotherapeutic that blocks the PD-1/PD-L1 interaction between tumor cells and activated T-cells (Figure 3). When the tumor cell is unable to interact with the activated T-cell, the immune system remains active, helping to prevent immunosuppression.

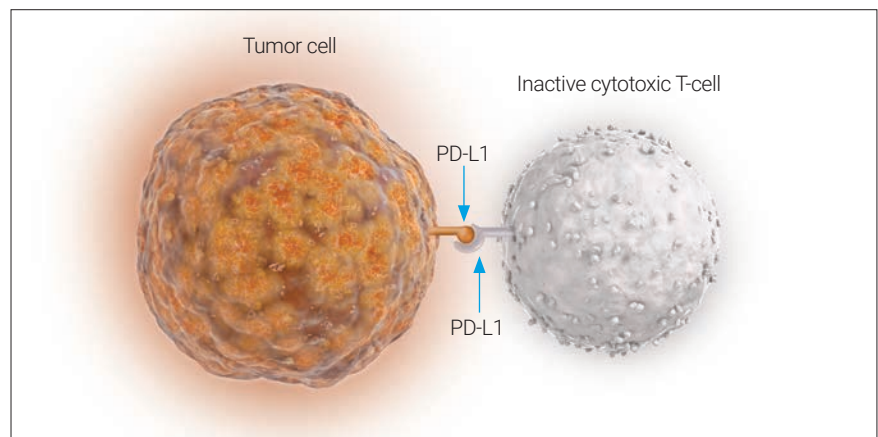
### PD-L1 IHC 22C3 pharmDx Detects PD-L1 in NSCLC Specimens

Tumor PD-L1 upregulation and detection on tumor cells is a biomarker for response to anti-PD-1 therapy. PD-L1 IHC 22C3 pharmDx was the only companion diagnostic used in the KEYTRUDA clinical trials (KEYNOTE-010 and KEYNOTE-024), which investigated the clinical efficacy of KEYTRUDA in patients with NSCLC. KEYTRUDA is a humanized monoclonal PD-1-blocking antibody.

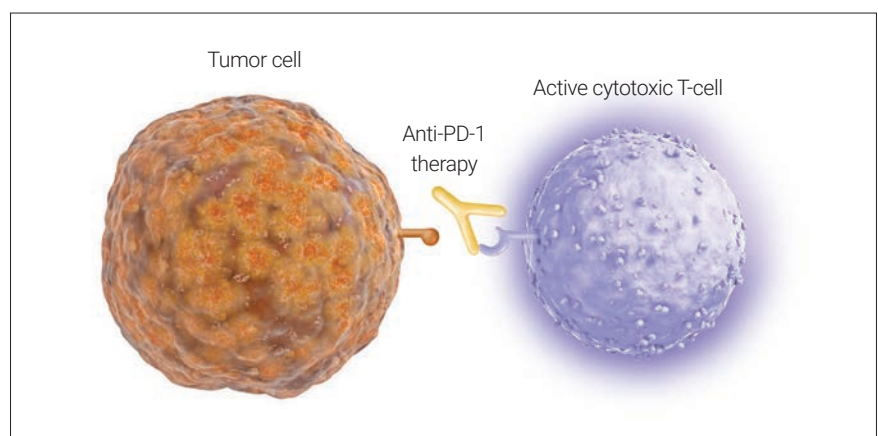




**Figure 1:** Inactivation of T-cells limits damage to normal tissue.



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



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

# PD-L1 IHC 22C3 pharmDx Overview

## What is PD-L1 IHC 22C3 pharmDx?

PD-L1 IHC 22C3 pharmDx is the only clinical trial-proven companion diagnostic indicated as an aid in identifying patients with NSCLC for treatment with KEYTRUDA® (pembrolizumab). PD-L1 IHC 22C3 pharmDx is a qualitative immunohistochemical (IHC) assay intended for use in the detection of PD-L1 in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tissue samples using Autostainer Link 48.

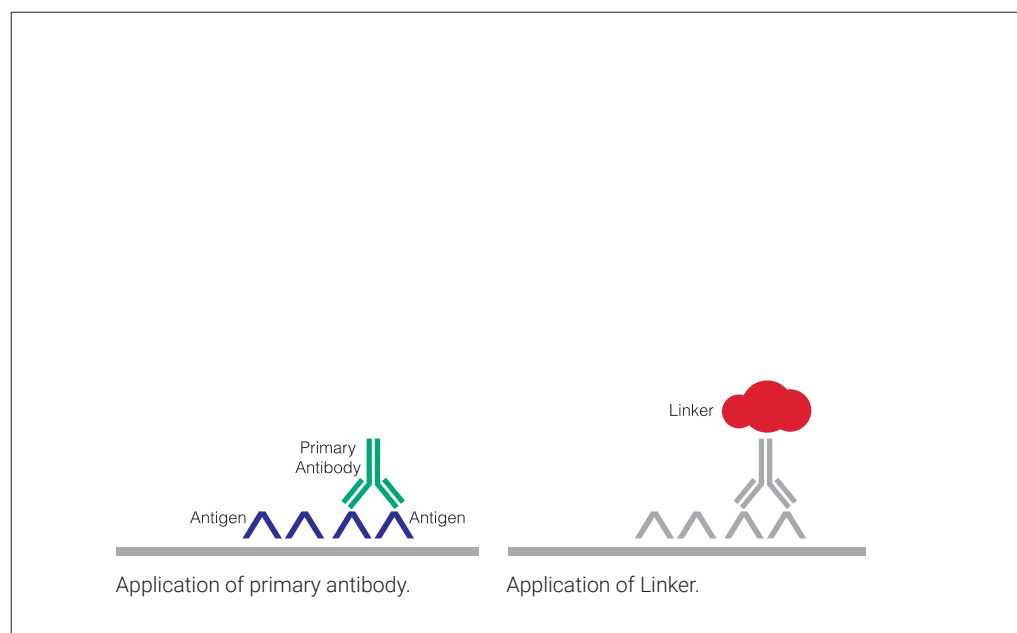
PD-L1 IHC 22C3 pharmDx was the only assay used in the KEYTRUDA clinical trials to determine the PD-L1 expression of patients with NSCLC.

## 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 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



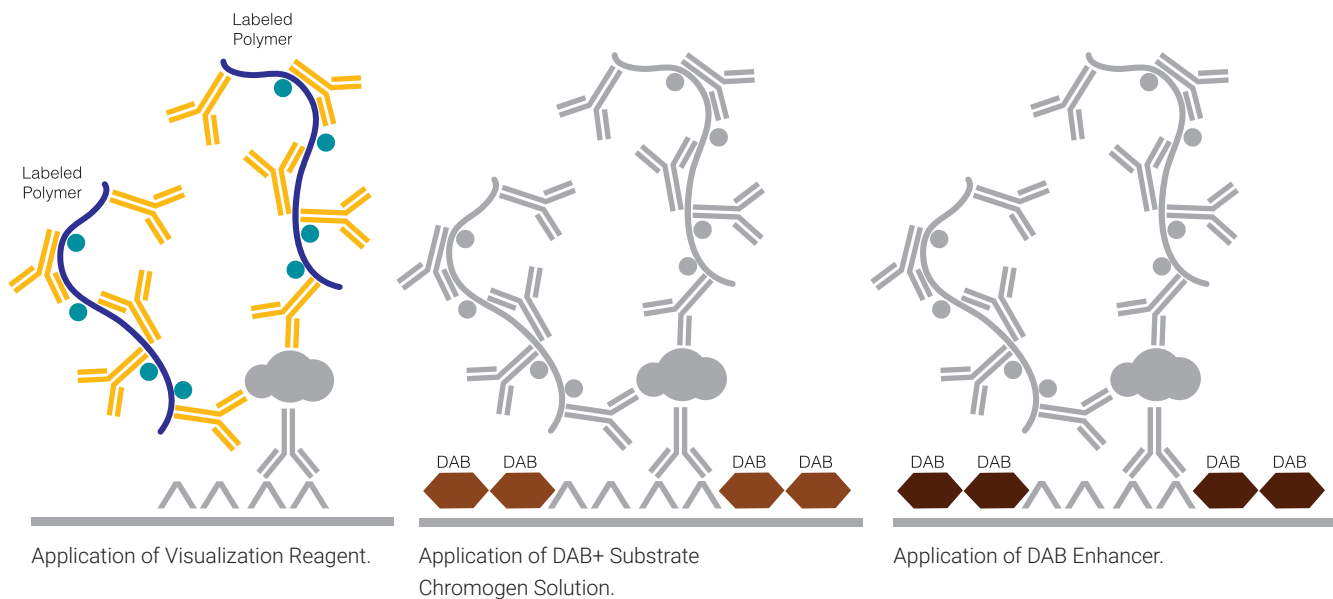
**Figure 5:** PD-L1 IHC 22C3 pharmDx components.

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\*

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

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



# Technical Considerations

Technical problems relating to the performance of PD-L1 IHC 22C3 pharmDx may stem from two areas: those involving specimen collection and preparation prior to performing the test, and those involving the actual performance of the test itself. Technical problems relating to the performance of the test generally are 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.

## 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 tissue in each staining run, in addition to the PD-L1 IHC 22C3 pharmDx Control Cell Line Slide.

Select positive and negative control tissue from fresh specimens of the same tumor indication as the patient specimen. Fix, process, and embed the control tissue in the same manner. Control tissues processed differently from the patient specimen validate reagent performance only and do not verify tissue preparation.

The ideal positive control tissue provides a complete dynamic representation of weak to moderate cell membrane staining. 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, as well as the surface epithelium should be negative.

## Tissue Processing

Formalin-fixed, paraffin-embedded 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. Studies 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 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 Fisherbrand Superfrost Plus slides, or Dako FLEX IHC microscope slides (Code K8020) 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 on Autostainer Link 48.

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

## EnVision FLEX Wash Buffer

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

## DAB+ Substrate-Chromogen Solution

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

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

## Controls to Assess Staining Quality

Include one PD-L1 IHC 22C3 pharmDx Control Cell Line Slide stained with the primary antibody in each staining run. For each set of test conditions, include positive and negative in-house control tissue stained with the primary antibody in each staining run. Use the Negative Control Reagent in place of the primary antibody on a sequential section of each patient specimen.

## 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 EnVision FLEX Target Retrieval Solution, Low pH, working solution to cover the tissue sections
- Preheat the Target Retrieval Solution, Low pH to 65 °C
- Immerse Autostainer racks containing mounted, FFPE tissue sections into the preheated Target Retrieval Solution, Low pH in PT Link tank. Incubate for 20 minutes at 97 °C
- When incubation has been completed and the temperature has cooled to 65 °C, remove each Autostainer slide rack with slides from the PT Link tank and immediately place the slides into a tank (e.g., PT Link Rinse Station, Code PT109) containing room temperature EnVision FLEX Wash Buffer working solution
- Leave Autostainer rack with slides in room temperature 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 EnVision FLEX Hematoxylin, Code K8008

## Mounting

Use non-aqueous permanent mounting media.

To minimize fading, store slides in the dark at room temperature (20–25 °C).

# Technical Checklist

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

Customer Name/Institution \_\_\_\_\_

Name and Title \_\_\_\_\_

Autostainer Link 48 Serial Number \_\_\_\_\_ Software Version \_\_\_\_\_

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

Additional observations or comments:

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# Clinical Interpretation Guidelines

## 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 scoring guidelines are reviewed on page 19. Before examining the patient specimen for PD-L1 staining, it is important to examine the controls to assess staining quality.

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

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 be assessed with every IHC run.

## Tissue Criteria

### Confirm the Presence of at Least 100 Viable Tumor Cells

A hematoxylin and eosin (H&E) stained section is recommended for the evaluation of an acceptable sample. PD-L1 IHC 22C3 pharmDx and the H&E staining should be performed on serial sections from the same paraffin block of the specimen.

Examine the H&E to determine if the specimen contains a minimum of 100 viable tumor cells.

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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.

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### Instructions for Patient Specimens with Less Than 100 Viable Tumor Cells

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

## Evaluating Controls

### 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 and the staining intensity. 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.

## Evaluating Controls



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

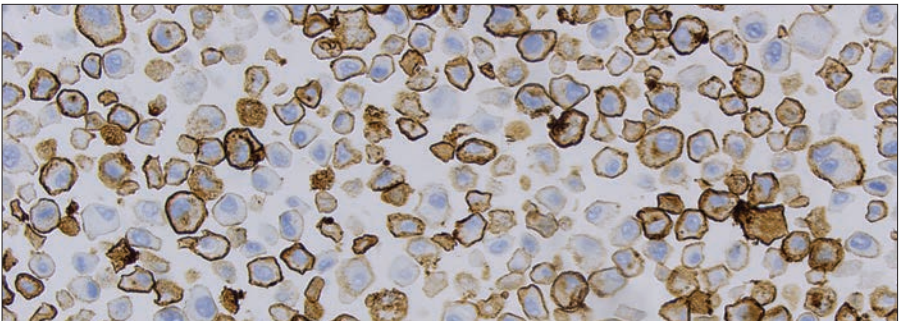
Evaluate the overall staining intensity using the following guide:

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):

- At least 70% of the cells contain cell membrane staining of at least 2+ average staining intensity
- Any background staining is less than 1+ staining intensity

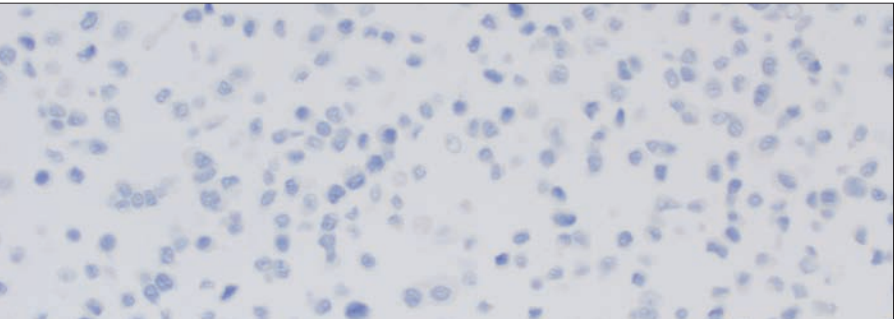


**Figure 7:** Positive cell pellet with acceptable staining of PD-L1 IHC 22C3 pharmDx Control Cell Line Slide (20x magnification).

### Negative Control Cell Pellet

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

- The majority of cells should demonstrate no staining. Note: The presence of 10 or fewer cells with distinct cell membrane staining is acceptable
- Any background staining is less than 1+ staining intensity

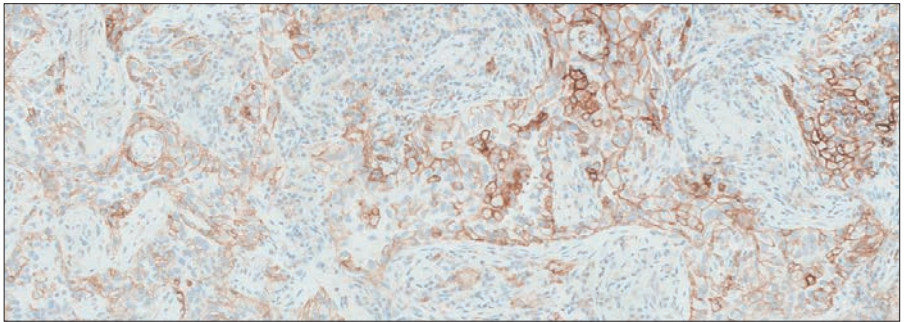


**Figure 8:** Negative cell pellet with no staining of PD-L1 IHC 22C3 pharmDx Control Cell Line Slide (20x magnification).

Do not use the Control Cell Line Slide as an aid in interpretation of patient results.

### Positive and Negative In-house Control Tissue (NSCLC)

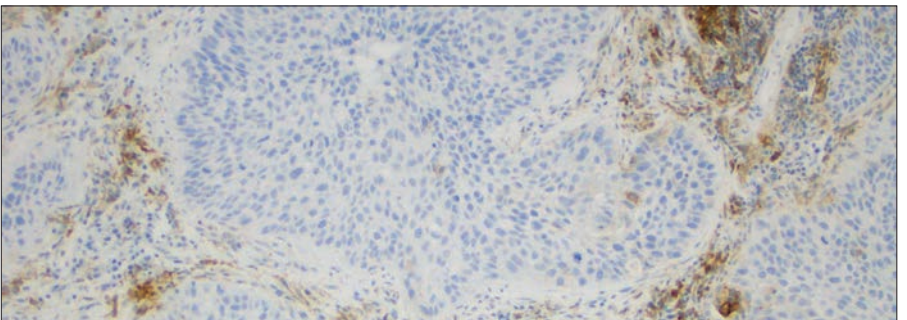
Examine the positive in-house NSCLC control tissue to determine that the tissues are correctly prepared and reagents are functioning properly. The ideal positive control tissue provides a complete dynamic representation of weak to moderate cell membrane staining (Figure 9). If staining of positive in-house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



**Figure 9:** Ideal positive in-house control tissue (10x magnification).

The ideal negative control tissue demonstrates no staining on tumor cells but contains tumor-associated macrophages/immune cells that express PD-L1 and offer an internal positive control (Figure 10, see next page). 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 negative in-house control tissue, results with the patient specimen should be considered invalid.

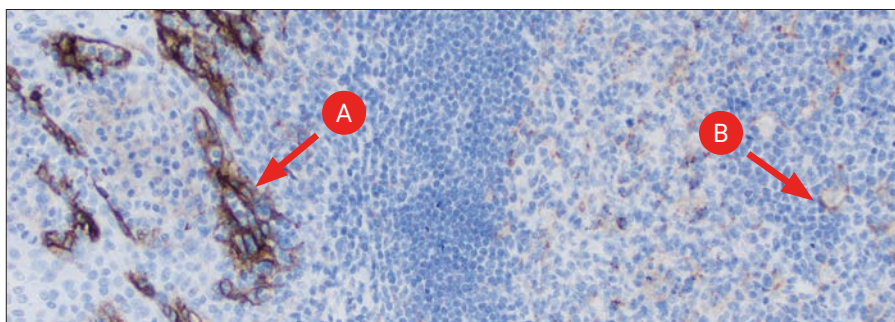


**Figure 10:** Ideal negative in-house control tissue (10x magnification).

### Optional Control Tissue

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 negative.



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

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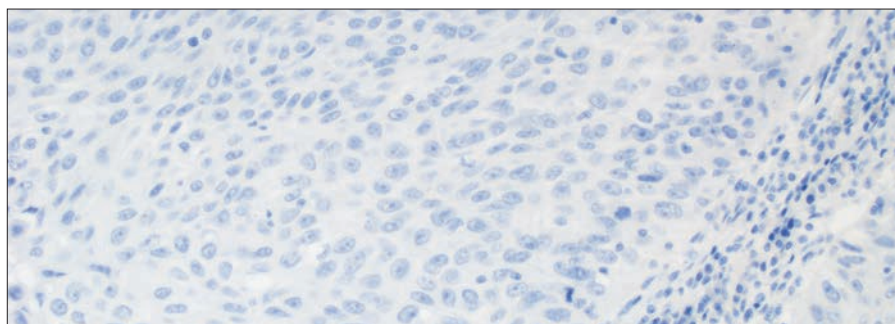
Do not use in-house control tissue as an aid in interpretation of patient results.

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### Negative Control Reagent (NCR)

Examine the positive and negative in-house controls stained with the NCR to determine that reagents are functioning properly. Absence of cell membrane staining of viable tumor cells is satisfactory (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.



**Figure 12:** Absence of cell membrane staining in NSCLC stained with Negative Control Reagent (20x magnification).

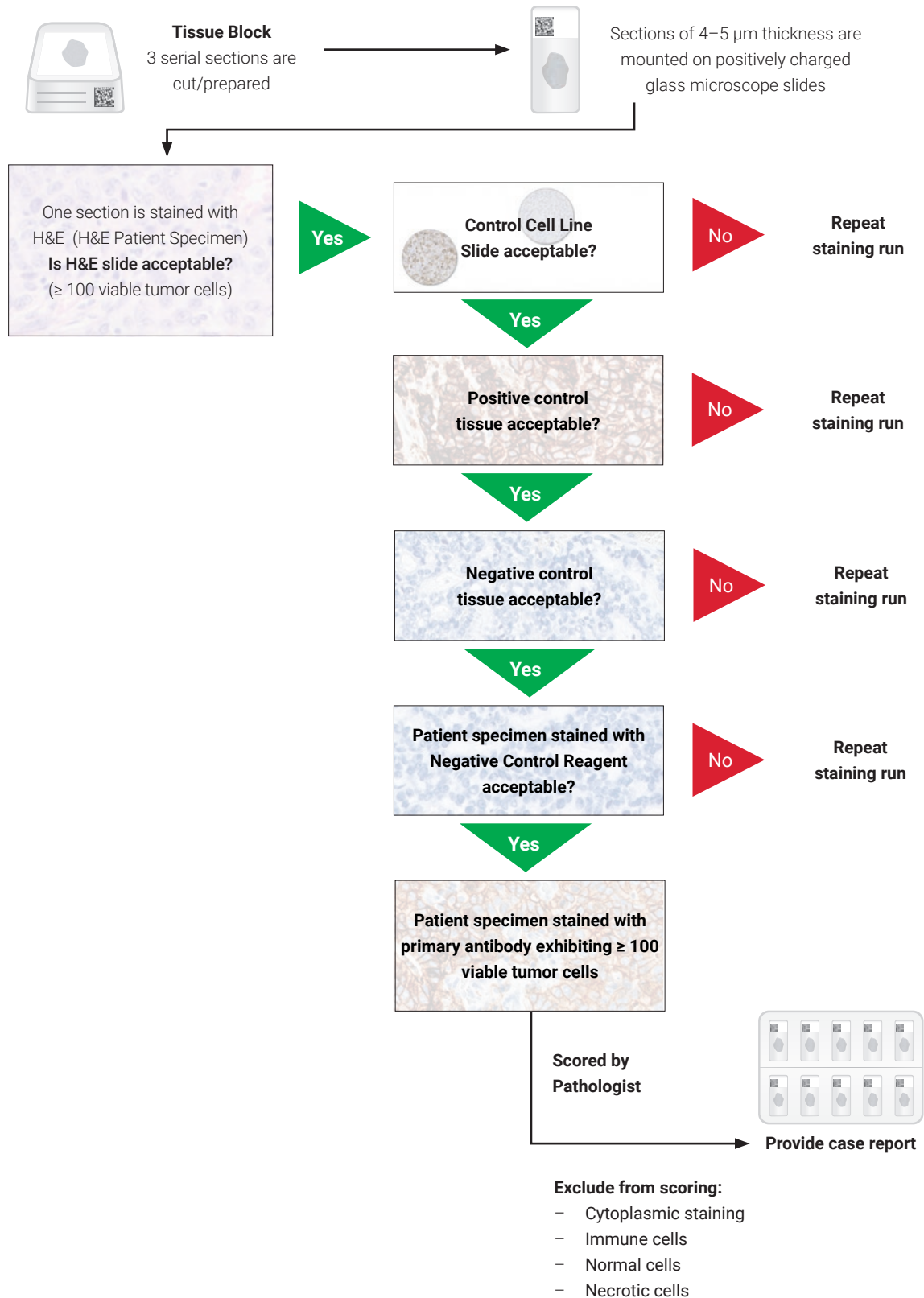
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The NCR indicates non-specific background staining and allows better interpretation of patient specimens stained with the primary antibody.

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# Verify Sample Acceptability



**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 ( $\geq 1+$ ) relative to all viable tumor cells present in the sample (positive and negative).

$$\text{TPS} = \frac{\text{\# PD-L1 positive tumor cells}}{\text{Total \# of PD-L1 positive + PD-L1 negative tumor cells}}$$

## Evaluation of PD-L1 Staining

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

Score only viable tumor cells. Exclude all other cells from scoring: infiltrating immune cells, normal cells, necrotic cells, and debris.

## Guidelines and Methods to Determine Tumor Proportion Score

- At low magnification, examine all well-preserved tumor areas. Evaluate overall areas of positive and negative 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 10x, 20x, and 40x, 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 positive tumor areas and negative tumor areas
    - Determining partial and complete membrane staining  $\geq 1+$
  - Calculate the Tumor Proportion Score by evaluating the percentage of PD-L1 positive 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**

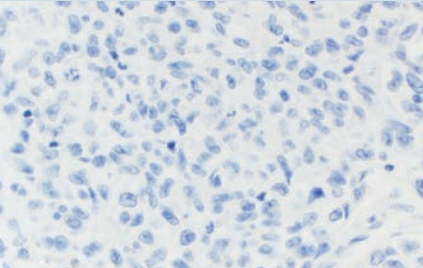
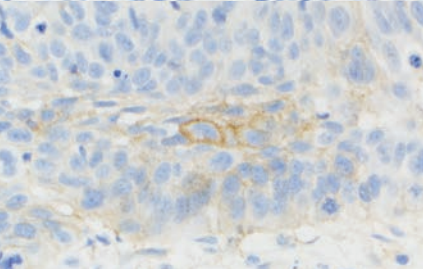
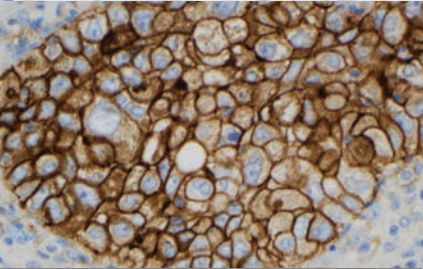
The following considerations can help distinguish tumor cells from immune cells:

- 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

# Scoring Guidelines

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

Table 1: TPS Expression Levels and Staining Characteristics

Expression Level	TPS	Staining Pattern	
No PD-L1 Expression	< 1%	Partial or complete cell membrane staining (≥ 1+) in < 1% of viable tumor cells	
PD-L1 Expression	1–49%	Partial or complete cell membrane staining (≥ 1+) in ≥ 1–49% of viable tumor cells	
High PD-L1 Expression	≥ 50%	Partial or complete cell membrane staining (≥ 1+) in ≥ 50% of viable tumor cells	

# 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 considering various staining patterns to determine the respective Tumor Proportion Scores.

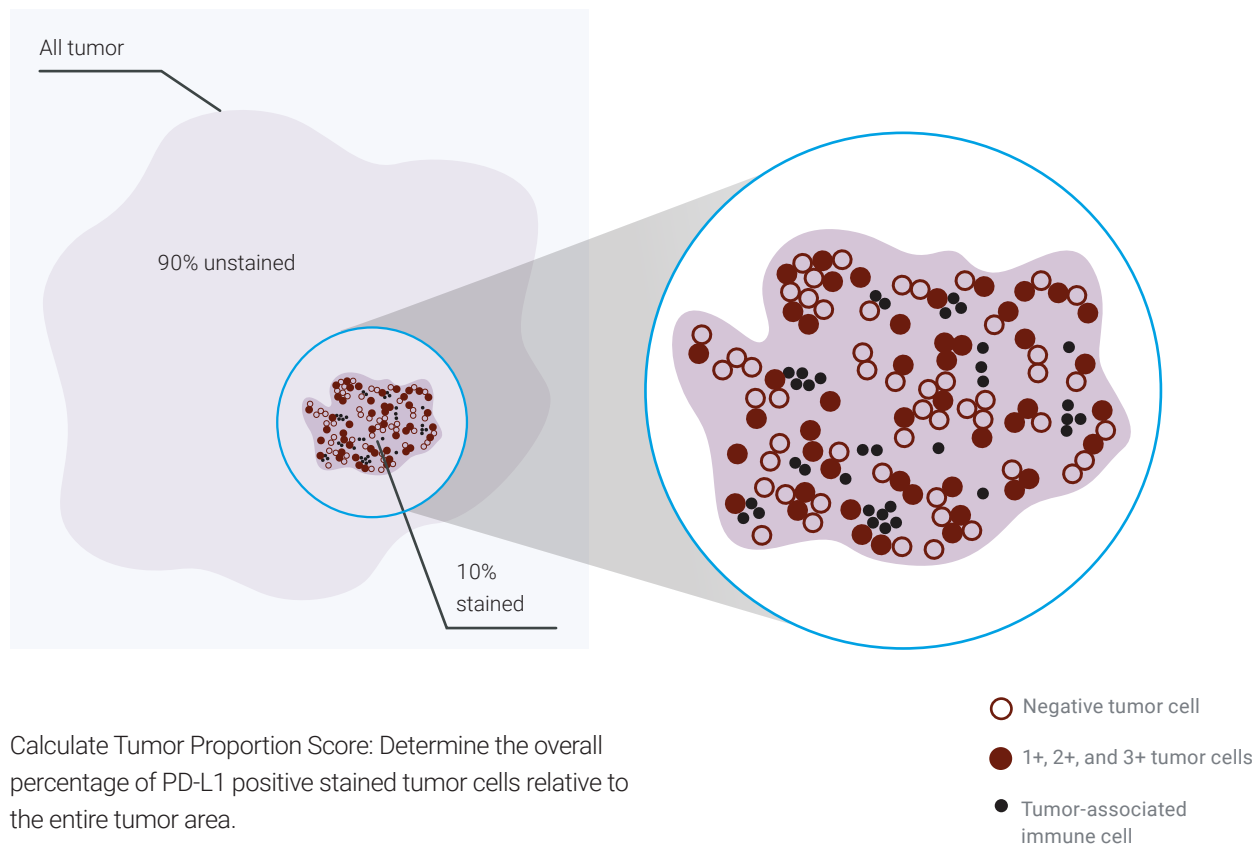
## Example 1: Calculation of Combined Positive Score in a Small Tumor Area With Staining

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

**Assessment:** 10% of area with staining, 90% of area without staining

At higher magnification: Evaluate the area of staining to estimate the percentage of tumor cells that are PD-L1 positive and PD-L1 negative.

**Assessment:** 50% of these cells are PD-L1 positive



Calculate Tumor Proportion Score: Determine the overall percentage of PD-L1 positive stained tumor cells relative to the entire tumor area.

**Assessment: Tumor Proportion Score (TPS):**

**10% x 50% = 5%**

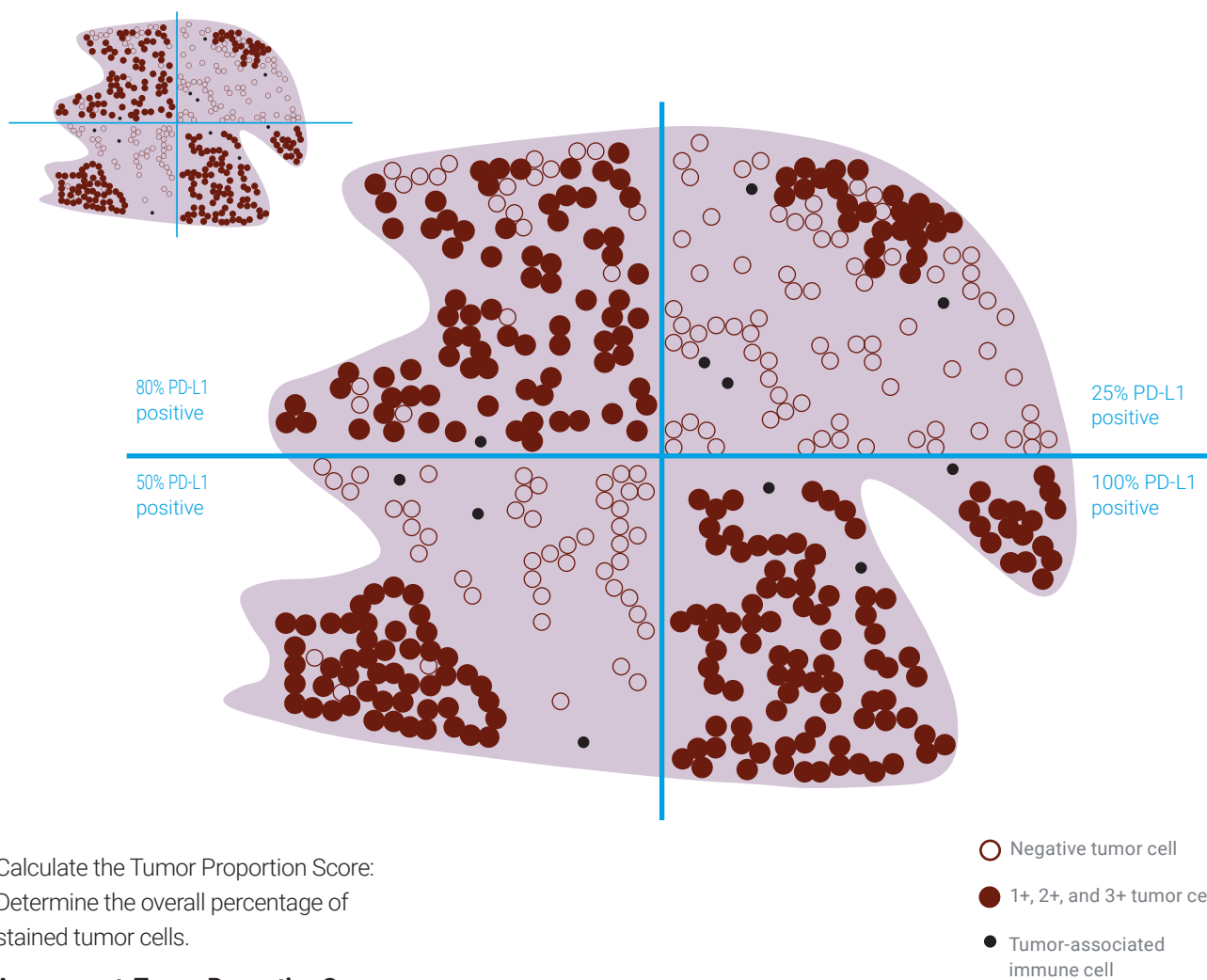
**Figure 14:** Example of tumor with small staining area.

## Example 2: Calculation of Tumor Proportion Score in a Heterogeneous Tumor Area

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

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

**Assessment:** Staining of tumor cells in each of the four respective sections: 80%, 25%, 50%, 100%



Calculate the Tumor Proportion Score:  
Determine the overall percentage of stained tumor cells.

**Assessment: Tumor Proportion Score:**  
 $(80\% + 25\% + 50\% + 100\%) / 4 = \geq 60\%$

**Figure 15:** Example with heterogeneous tumor area.

# Identifying Patients with NSCLC for Treatment

PD-L1 IHC 22C3 pharmDx is the only clinical trial-proven companion diagnostic indicated as an aid in identifying patients with NSCLC for treatment with KEYTRUDA® (pembrolizumab).

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

The clinical validity of PD-L1 IHC 22C3 pharmDx in identifying high PD-L1 expression (TPS  $\geq$  50%) in previously untreated patients with 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  $\geq$  50% were included in the KEYNOTE-024 study. Efficacy of KEYTRUDA treatment in patients selected by PD-L1 IHC 22C3 pharmDx is presented in the Clinical Performance Evaluation section on pages 66–70.

Table 2: PD-L1 Prevalence<sup>a</sup> in Patients with NSCLC<sup>b</sup> Screened for KEYNOTE-024<sup>c</sup>

PD-L1 Expression	TPS < 1%	TPS 1–49%	TPS $\geq$ 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 chemotherapy (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 NSCLC (Second-line and Beyond)

The clinical validity of PD-L1 IHC 22C3 pharmDx in identifying PD-L1 expression (TPS  $\geq$  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 NSCLC were tested for PD-L1 expression using PD-L1 IHC 22C3 pharmDx. Only patients with TPS  $\geq$  1% were included in the KEYNOTE-010 study. Efficacy of KEYTRUDA treatment in patients selected by PD-L1 IHC 22C3 pharmDx is presented in the Clinical Performance Evaluation section on pages 66–70.

Table 3: PD-L1 Prevalence<sup>d</sup> in Patients with NSCLC<sup>e</sup> Screened for KEYNOTE-010<sup>f</sup>

PD-L1 Expression	TPS < 1%	TPS 1–49%	TPS $\geq$ 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.

PD-L1 IHC 22C3 pharmDx  
Testing Algorithm

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

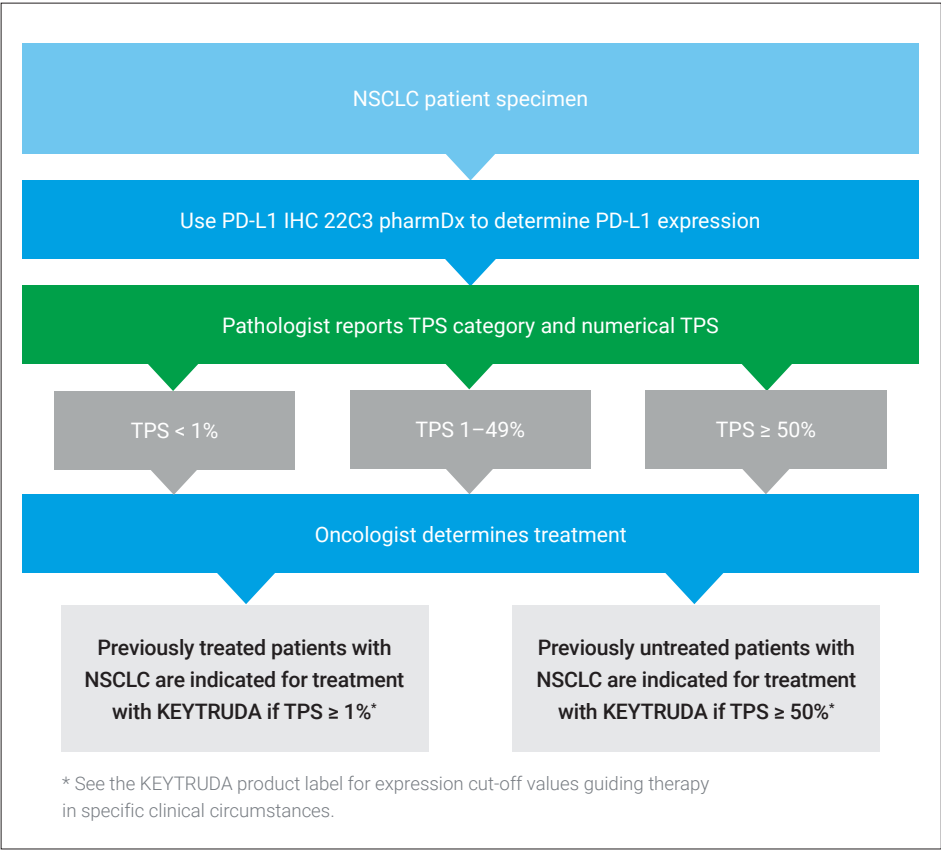


Figure 16: Testing algorithm for PD-L1 IHC 22C3 pharmDx.



## Reporting Results

Suggested information to include when reporting results for non-small cell lung cancer 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 ( $\geq 100$  cells): ☐

### PD-L1 IHC 22C3 pharmDx Result to Treating Physician

Tumor Proportion Score: \_\_\_\_\_

TPS  $\geq 50\%$  (High PD-L1 expression): ☐ TPS 1–49% (PD-L1 expression): ☐ TPS  $< 1\%$  (No PD-L1 expression): ☐

# PD-L1 Staining Characteristics

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

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

- A minimum of 100 viable tumor cells are present for evaluation
- The appropriate cells are evaluated—only viable tumor cells should be scored
- The proper cellular localization is identified—only the 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 4x, 10x, 20x, and 40x magnifications (at a minimum) are appropriate.

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

- Membrane staining of tumor cells at all intensities 1–3+ should be included
- Partial and/or complete membrane 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

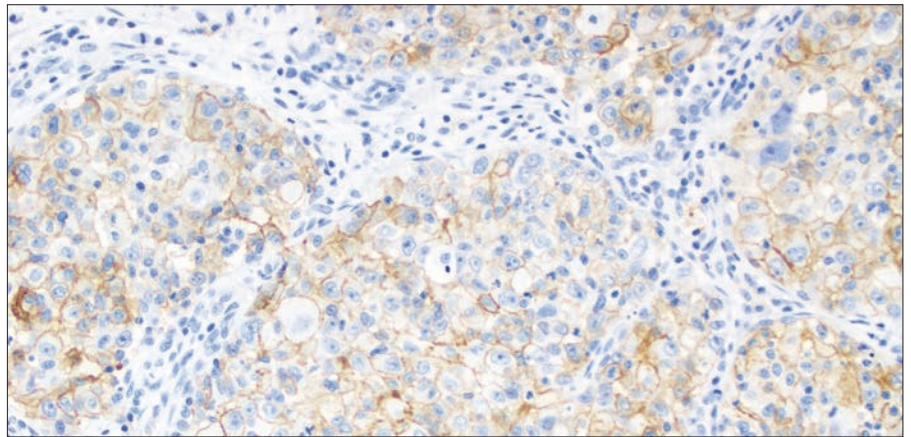
The following pages provide guidance on various staining characteristics.

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

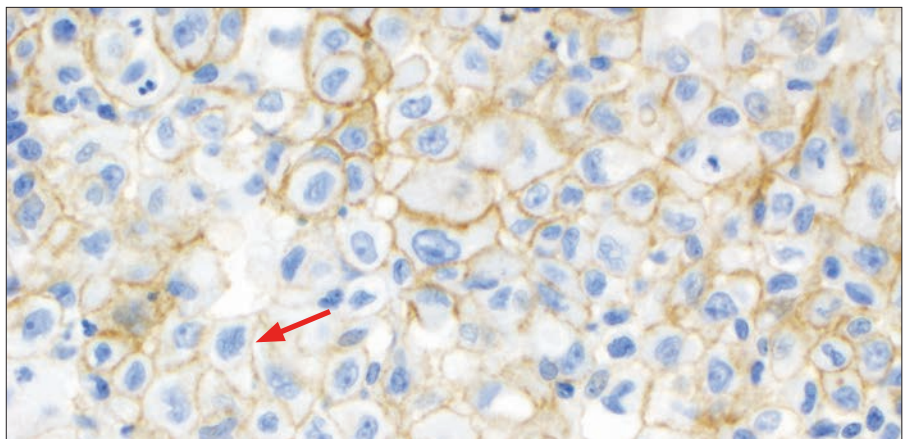
### Perceptible and Convincing Membrane Staining

Scoring should include any perceptible and convincing membrane staining ( $\geq 1+$ ) at any magnification.

Review at higher magnification may be needed to confirm perceptible and convincing membrane staining.



**Figure 17a:** NSCLC stained with PD-L1 primary antibody exhibiting weak membrane staining of tumor cells (10x magnification).



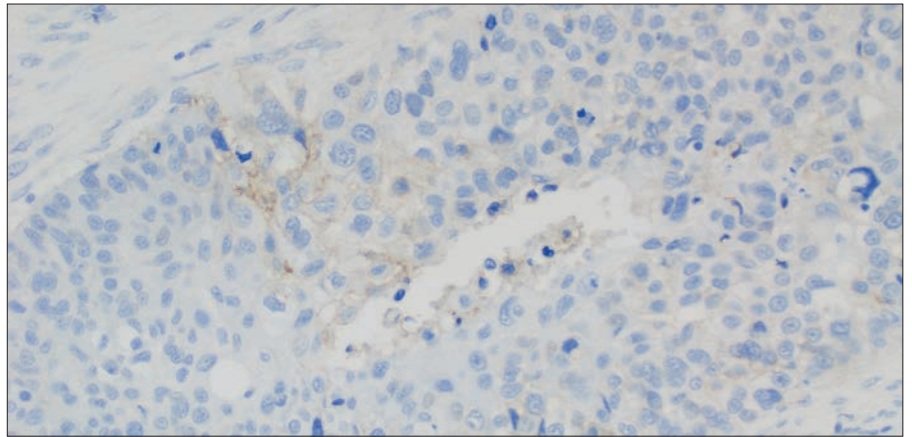
**Figure 17b:** NSCLC stained with PD-L1 primary antibody exhibiting weak but perceptible and convincing membrane staining of tumor cells (arrow) (40x magnification).

### Key point

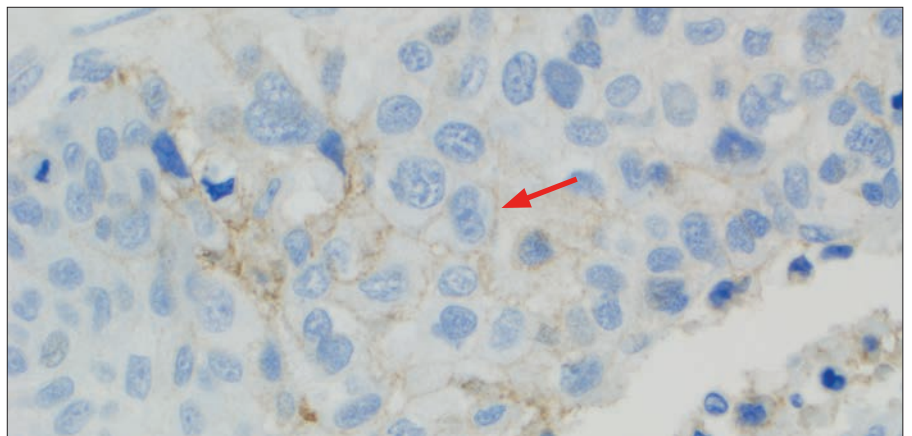
Any perceptible and convincing membrane staining of tumor cells ( $\geq 1+$ ) should be included in the TPS

### Weak Acceptable Membrane Staining

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



**Figure 18a:** NSCLC stained with PD-L1 primary antibody exhibiting weak but perceptible and convincing membrane staining of tumor cells (20x magnification).



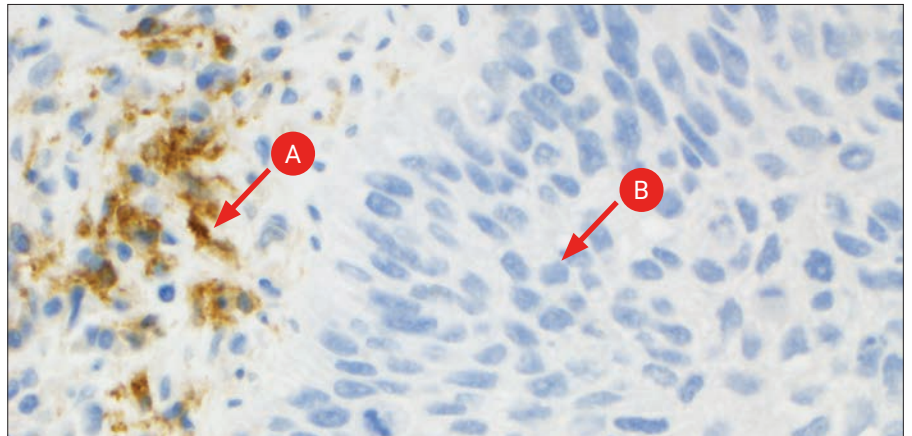
**Figure 18b:** NSCLC stained with PD-L1 primary antibody exhibiting weak but perceptible and convincing membrane staining of tumor cells (arrow) (40x magnification).

### Key point

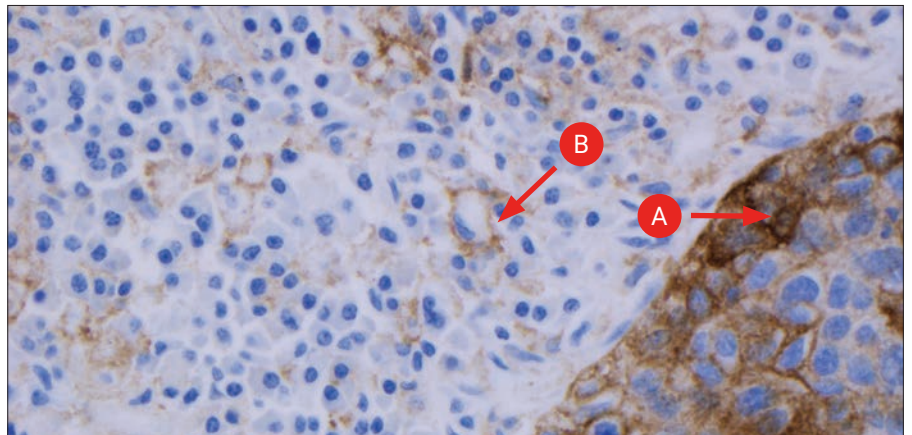
Weak but perceptible and convincing 1+ membrane staining of tumor cells should be included in the TPS

### Distinguishing Tumor Cells from Tumor-Associated Immune Cells (TAIC)

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



**Figure 19:** NSCLC 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 (20x magnification).



**Figure 20:** NSCLC 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 (20x magnification).

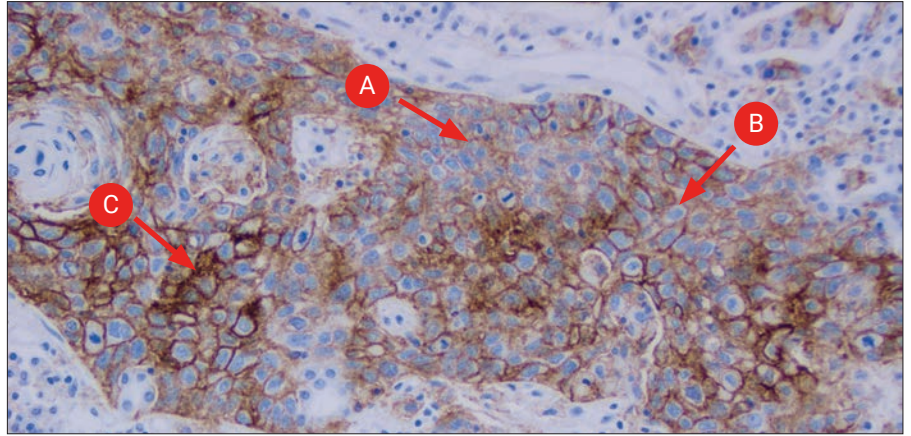
#### Key point

Staining of TAIC should be excluded from the TPS



### Heterogeneous Staining Intensities

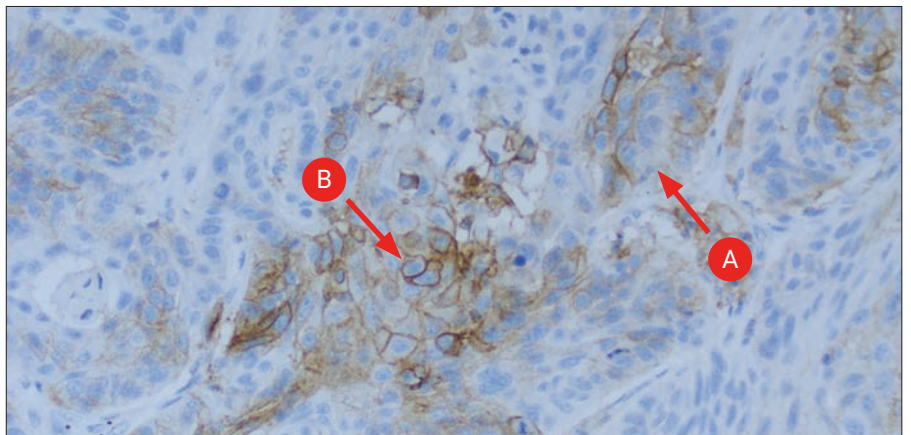
Membrane staining of PD-L1 on NSCLC specimens is often heterogeneous with various staining intensities (1–3+).



**Figure 21:** NSCLC 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) (20x magnification).

#### Key point

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



**Figure 22:** NSCLC 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) (20x magnification).

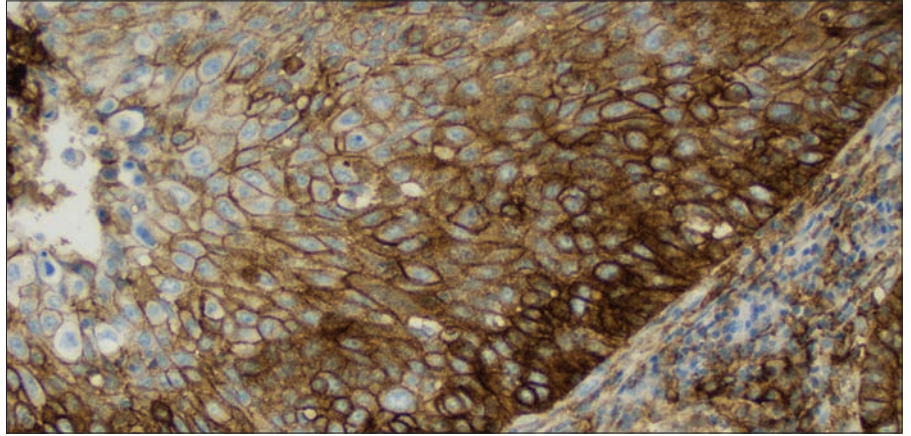
#### Key point

Partial and/or complete membrane staining of tumor cells should be included in the TPS



### Cytoplasmic and Membrane Staining

PD-L1 staining can exhibit cytoplasmic and/or membrane staining. Cytoplasmic staining should be excluded from the TPS scoring assessment.



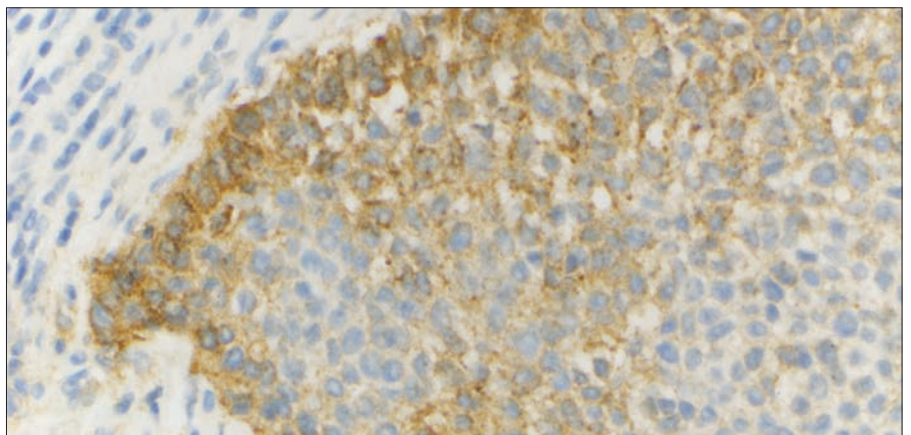
**Figure 23:** NSCLC stained with PD-L1 primary antibody exhibiting strong cytoplasmic and membrane staining of tumor cells (20x magnification).

#### 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.



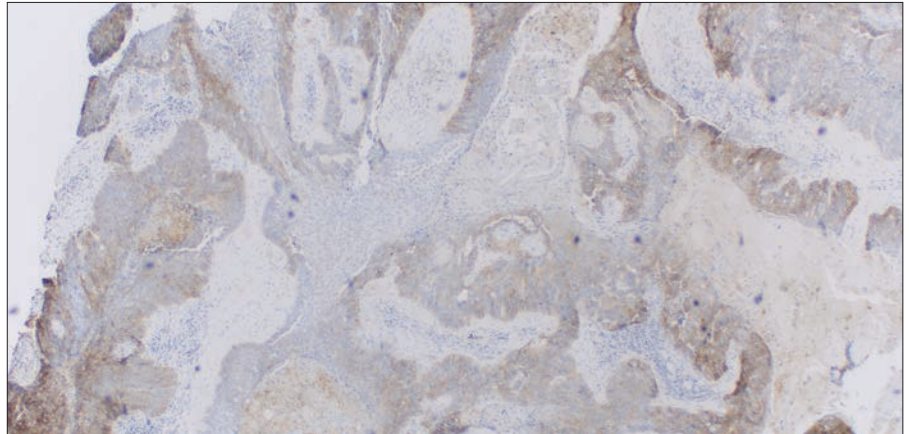
**Figure 24:** NSCLC specimen stained with PD-L1 primary antibody with the majority of tumor cells exhibiting a granular pattern of perceptible and convincing membrane staining (20x magnification).

#### Key point

Granular staining of tumor cells must be perceptibly and convincingly membranous 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.



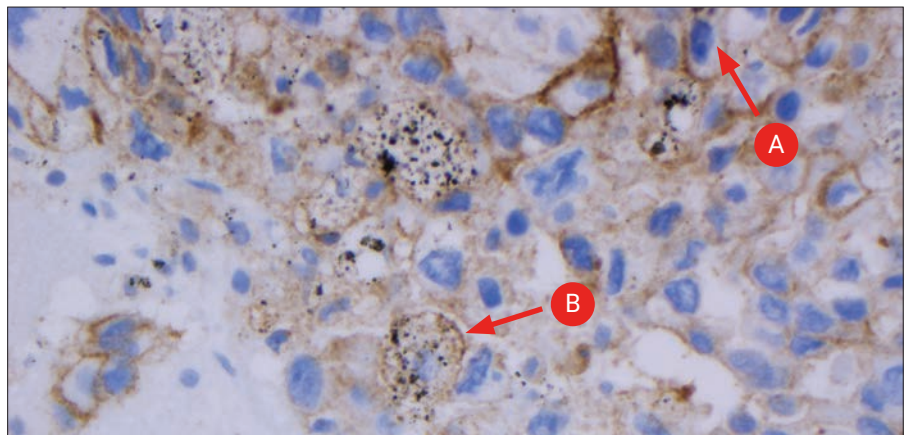
**Figure 25:** NSCLC stained with PD-L1 primary antibody exhibiting a patchy membrane staining pattern (10x magnification).

### 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 26:** NSCLC 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 (20x magnification)

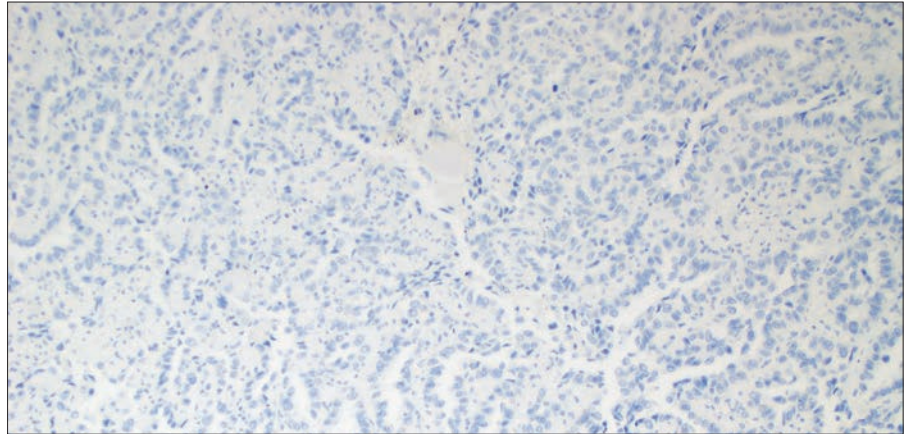
### Key point

Anthracotic pigment should be disregarded

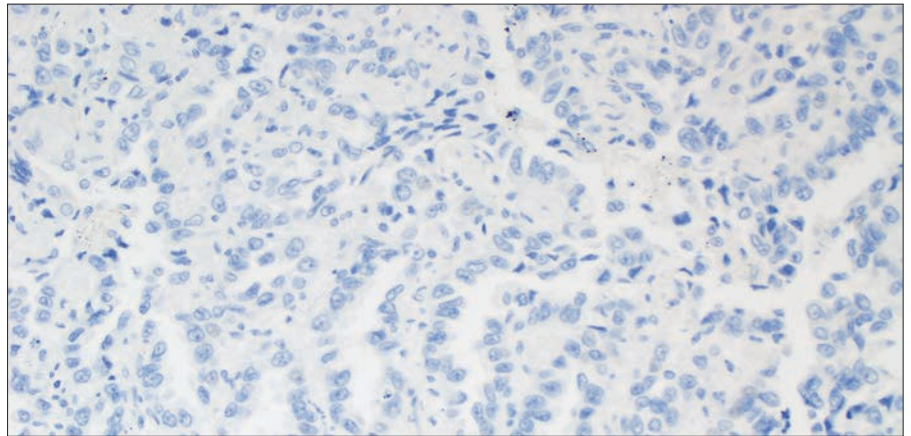


PD-L1 IHC 22C3 pharmDx  
TPS < 1% Case Examples

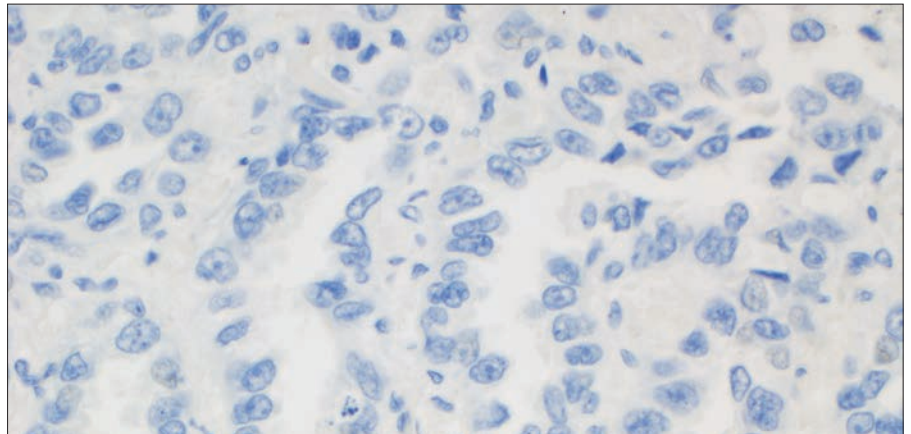
**Case 1: TPS < 1%**



**Figure 27a:** 10x magnification



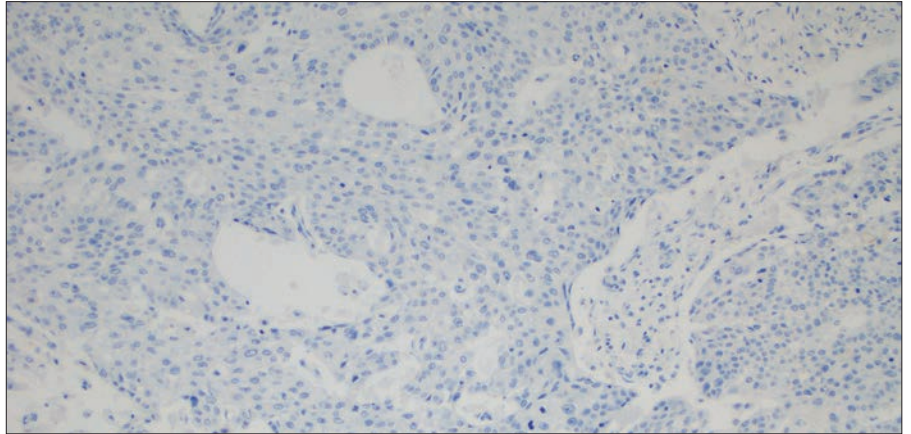
**Figure 27b:** 20x magnification



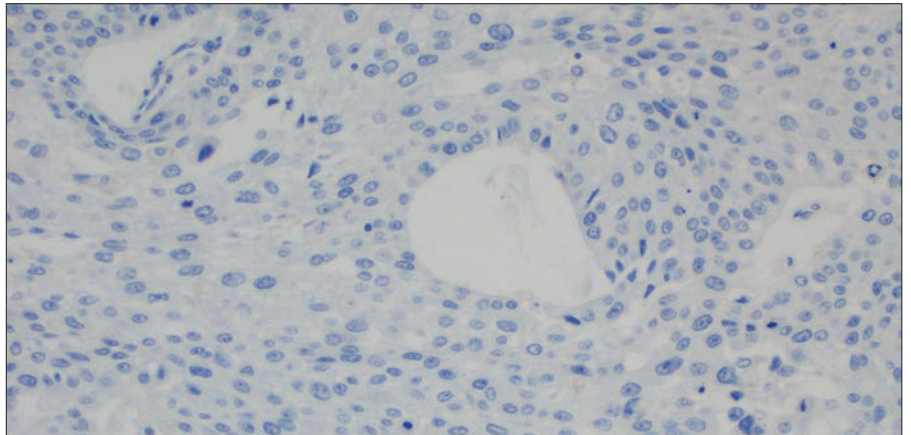
**Figure 27c:** 40x magnification

**Figure 27a–27c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS < 1%.

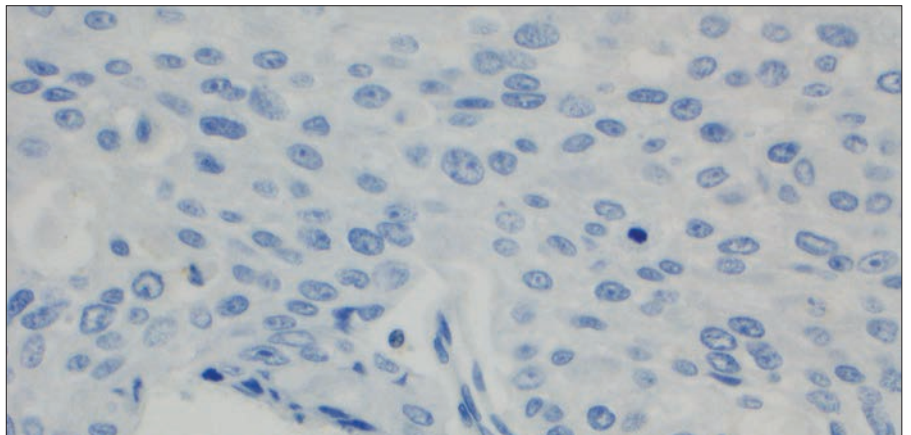
## Case 2: TPS < 1%



**Figure 28a:** 10x magnification



**Figure 28b:** 20x magnification

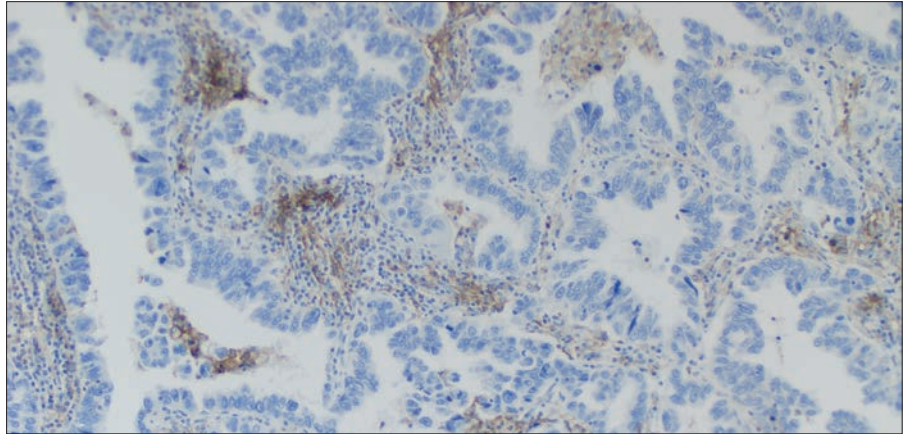


**Figure 28c:** 40x magnification

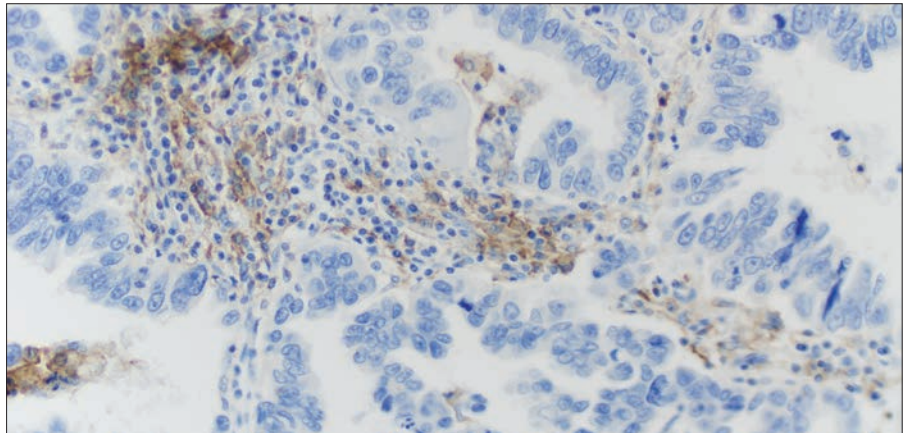
**Figure 28a–28c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS < 1%.



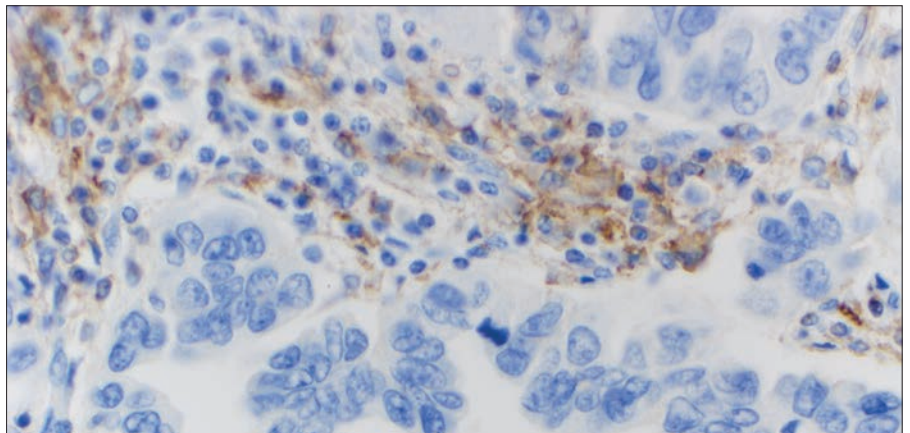
**Case 3: TPS < 1%**



**Figure 29a:** 10x magnification



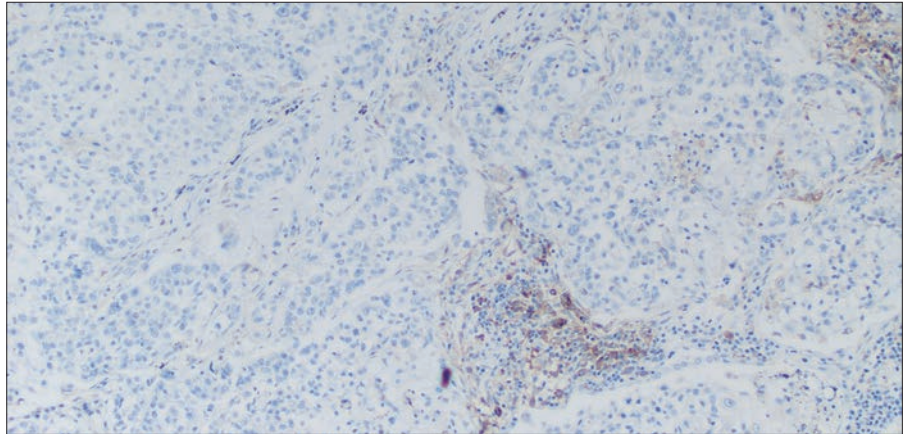
**Figure 29b:** 20x magnification



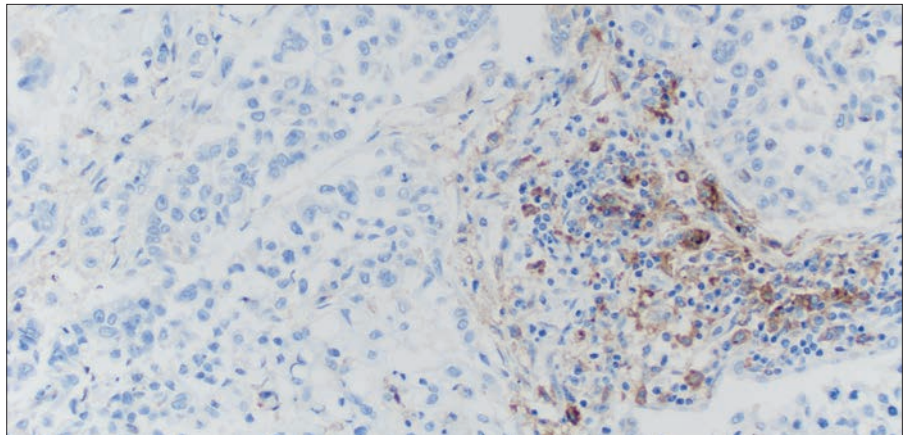
**Figure 29c:** 40x magnification

**Figure 29a–29c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS < 1%. TAIC are staining, but should be excluded from scoring.

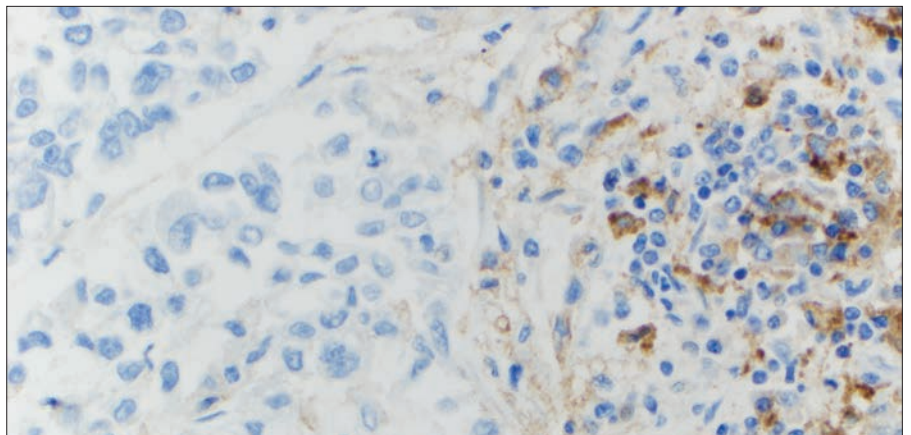
**Case 4 : TPS < 1%**



**Figure 30a:** 10x magnification



**Figure 30b:** 20x magnification



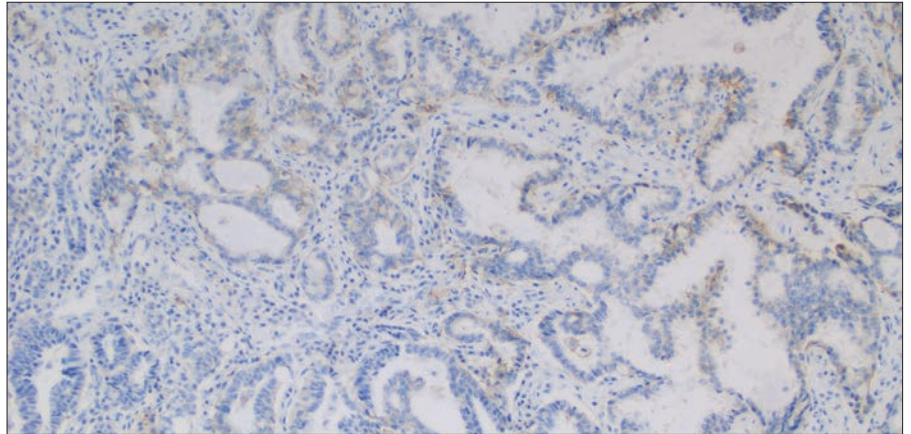
**Figure 30c:** 40x magnification

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

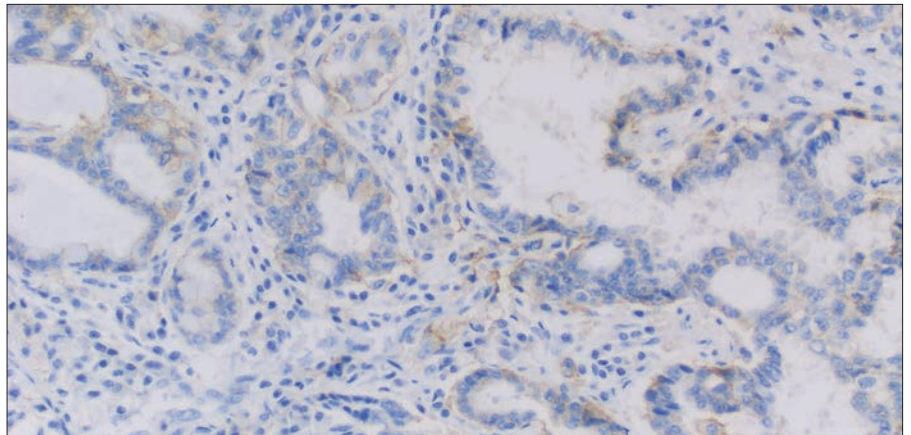


PD-L1 IHC 22C3 pharmDx  
TPS 1–49% Case Examples

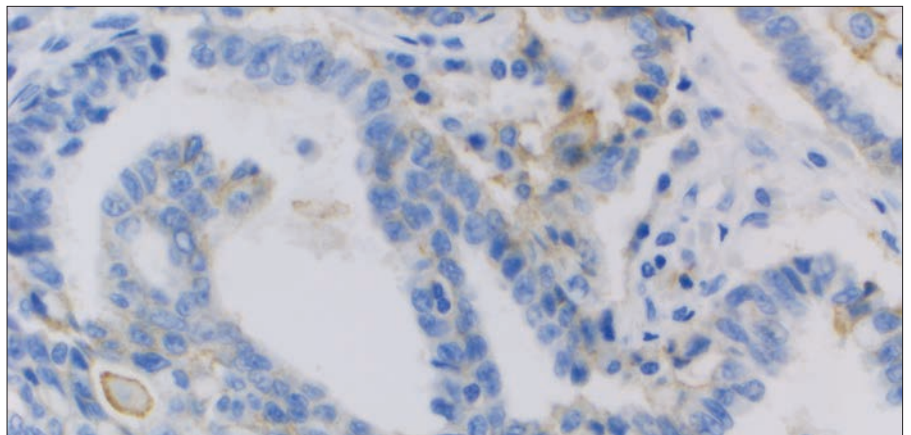
**Case 5: TPS 1–49%**



**Figure 31a:** 10x magnification



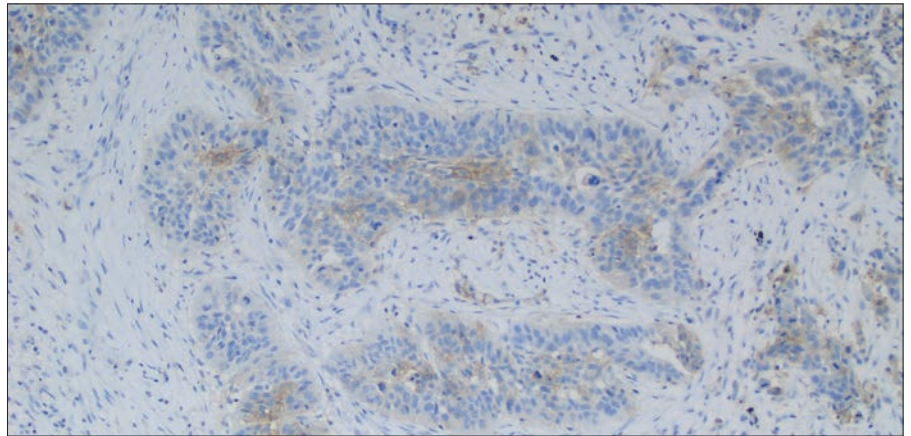
**Figure 31b:** 20x magnification



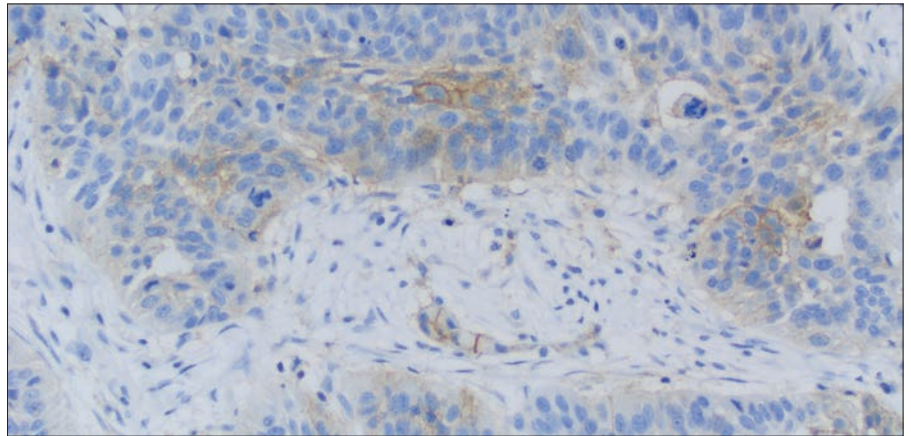
**Figure 31c:** 40x magnification

**Figure 31a–31c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS 1–49%.

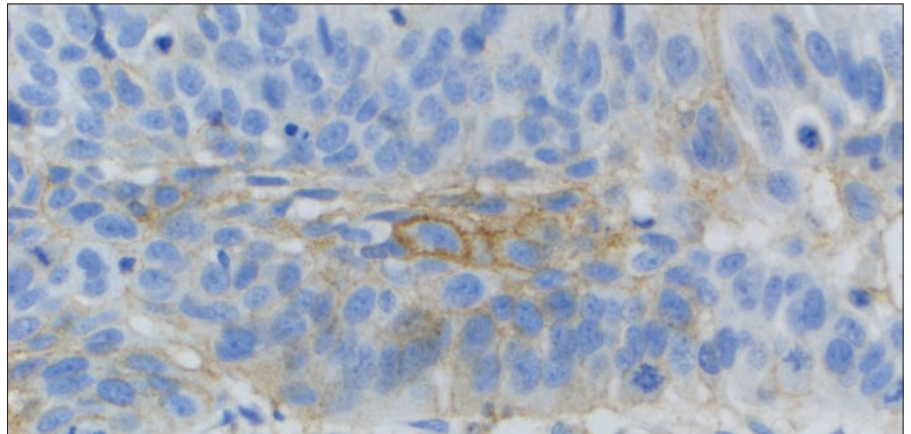
**Case 6: TPS 1–49%**



**Figure 32a:** 10x magnification



**Figure 32b:** 20x magnification

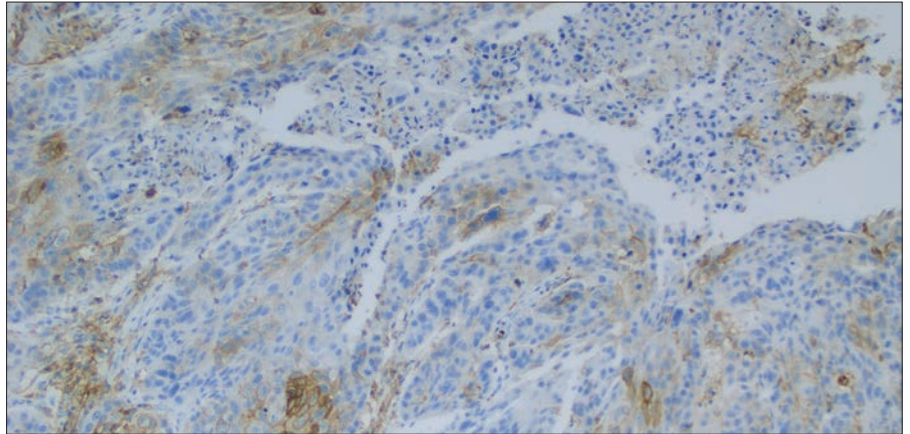


**Figure 32c:** 40x magnification

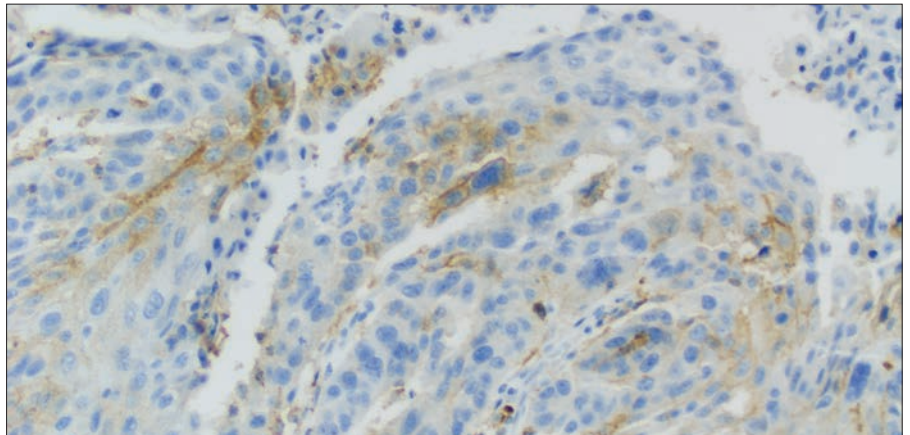
**Figure 32a–32c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS 1–49%.



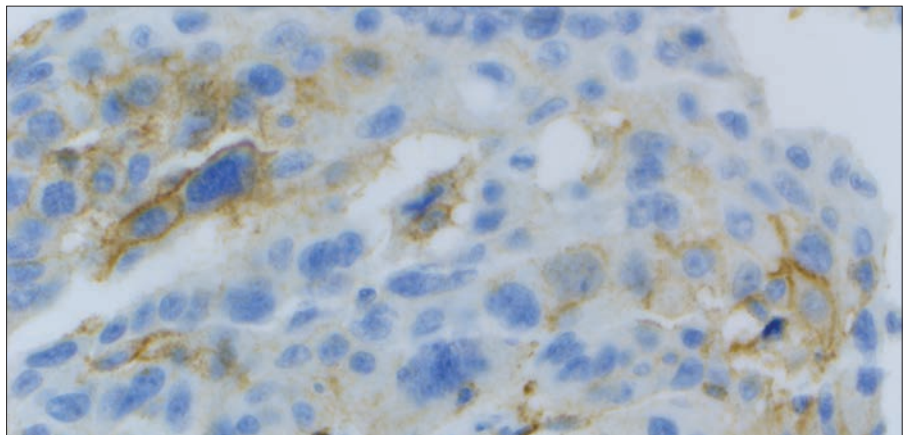
### Case 7 TPS 1–49%



**Figure 33a:** 10x magnification



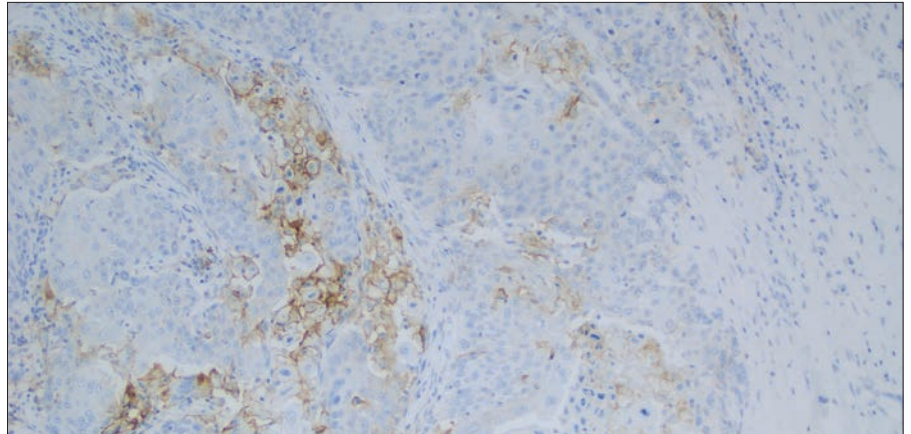
**Figure 33b:** 20x magnification



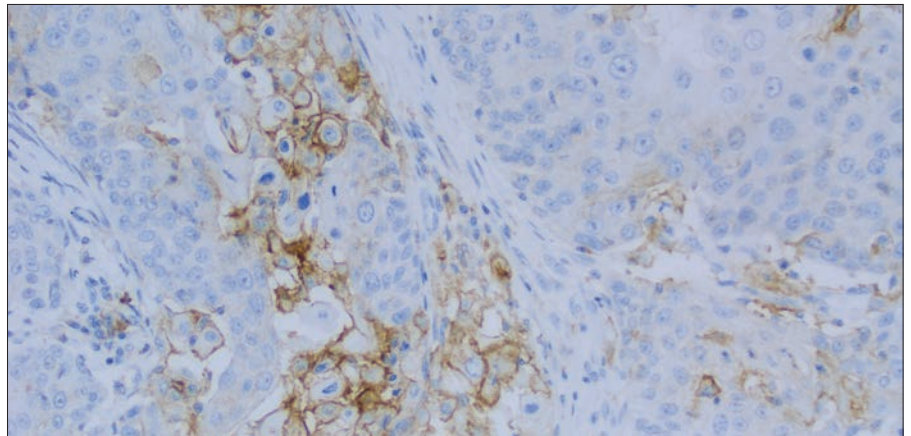
**Figure 33c:** 40x magnification

**Figure 33a–33c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS 1–49%.

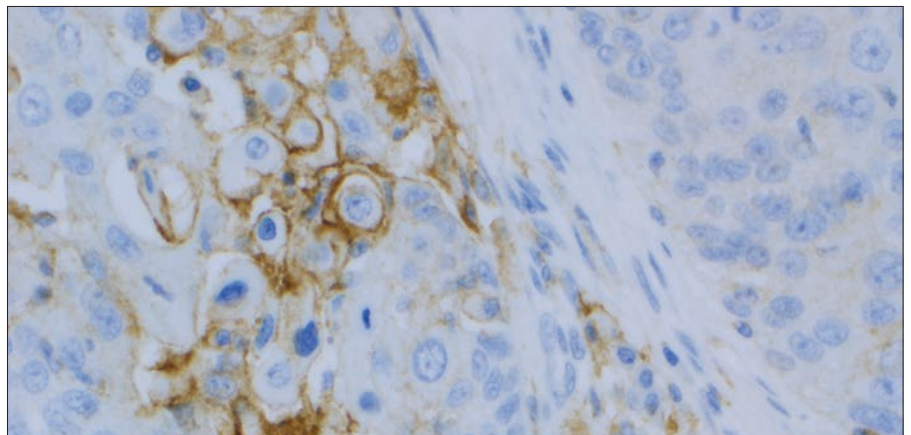
**Case 8: TPS 1–49%**



**Figure 34a:** 10x magnification



**Figure 34b:** 20x magnification



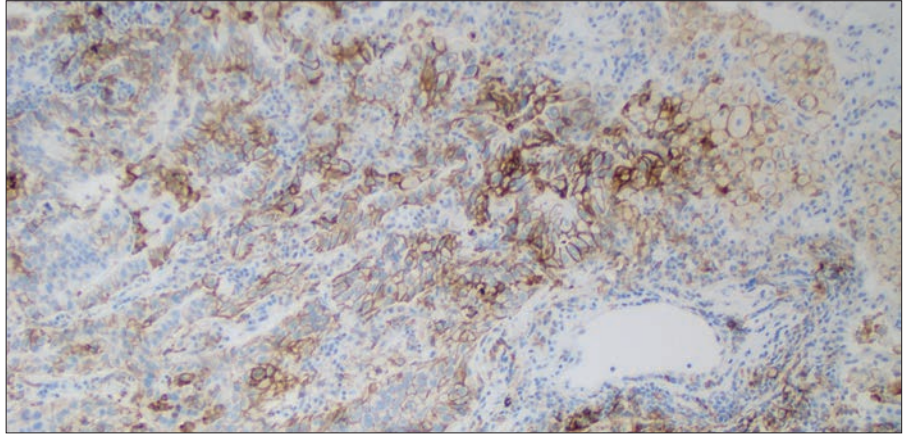
**Figure 34c:** 40x magnification

**Figure 34a–34c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS 1–49%.

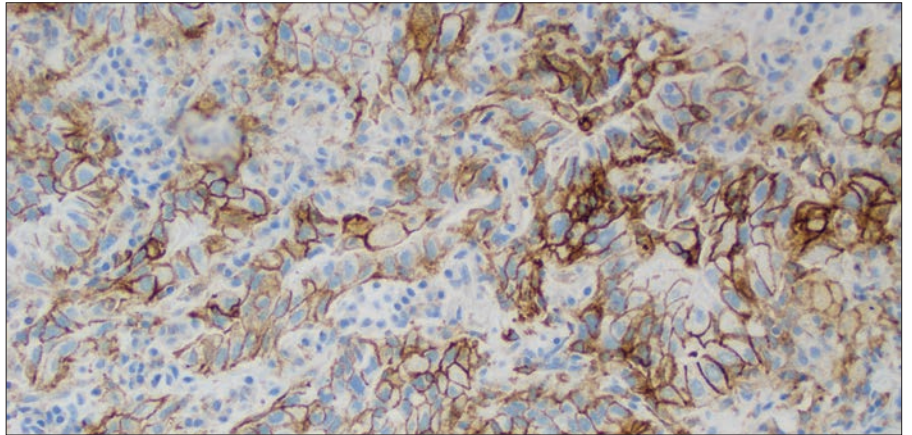


PD-L1 IHC 22C3 pharmDx  
TPS  $\geq$  50% Case Examples

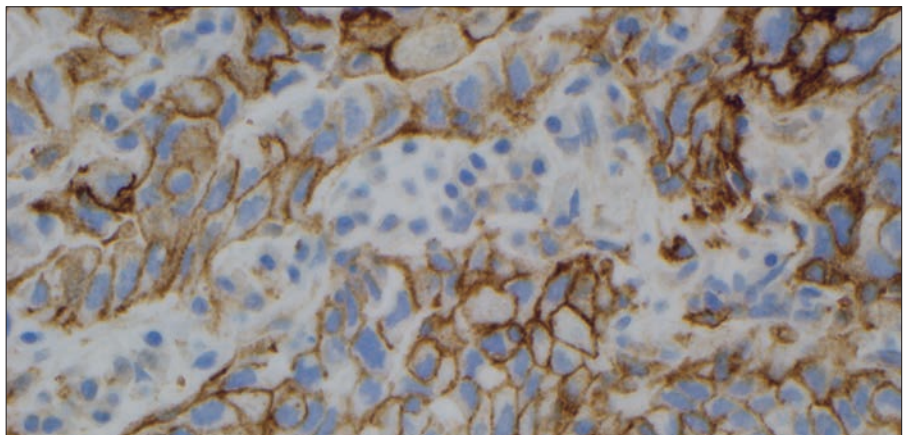
**Case 9: TPS  $\geq$  50%**



**Figure 35a:** 10x magnification



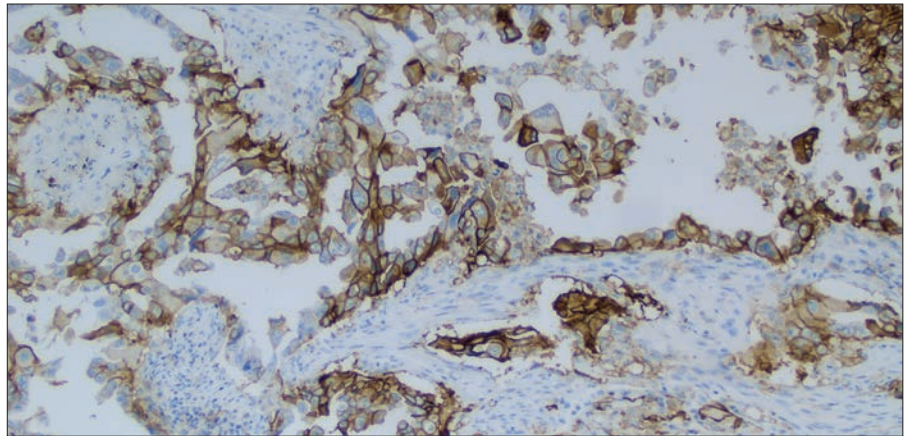
**Figure 35b:** 20x magnification



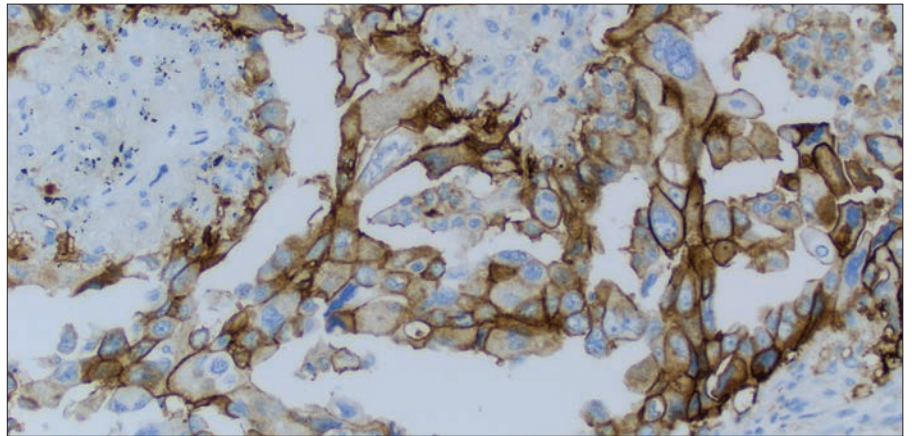
**Figure 35c:** 40x magnification

**Figure 35a–35c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS  $\geq$  50%.

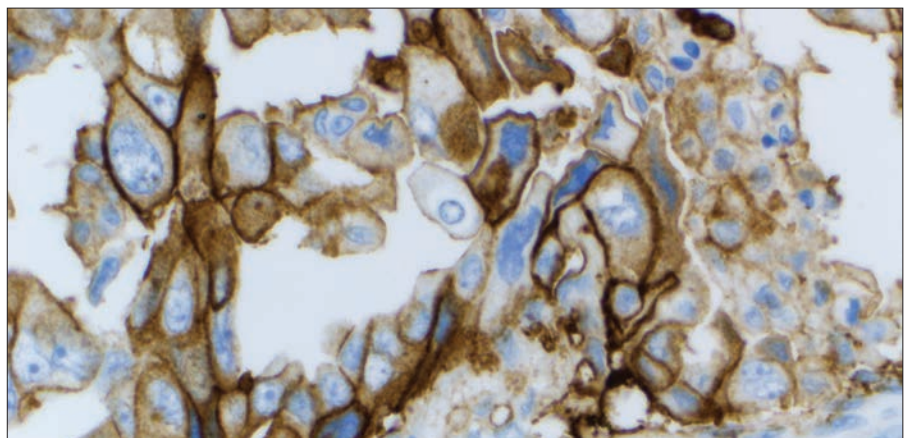
**Case 10: TPS  $\geq$  50%**



**Figure 36a:** 10x magnification



**Figure 36b:** 20x magnification

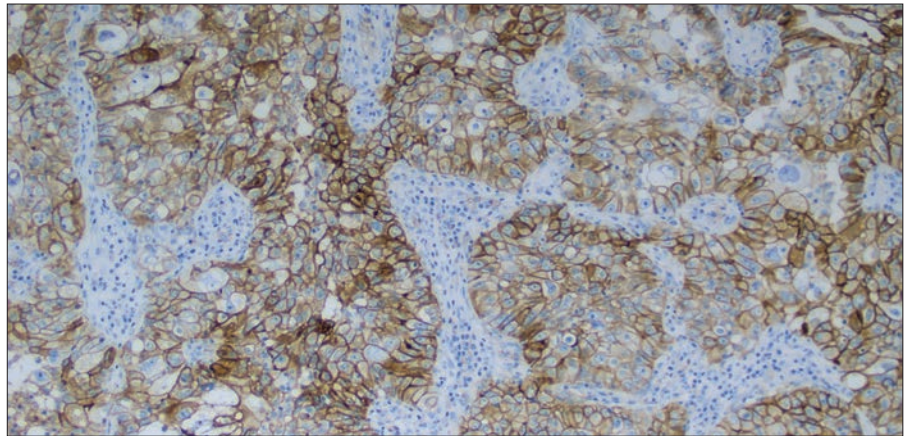


**Figure 36c:** 40x magnification

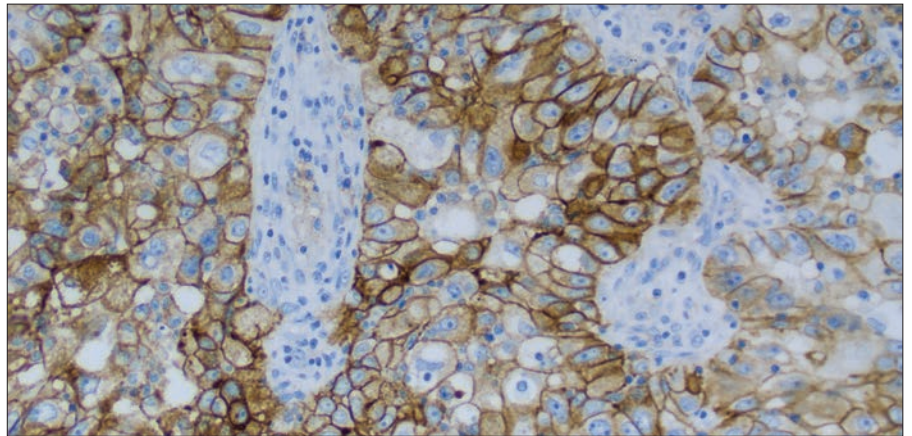
**Figure 36a–36c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS  $\geq$  50%.



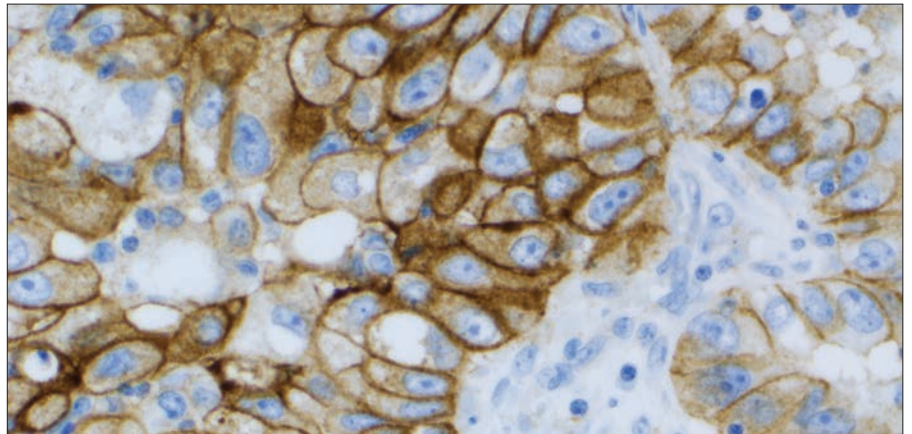
**Case 11: TPS  $\geq$  50%**



**Figure 37a:** 10x magnification



**Figure 37b:** 20x magnification

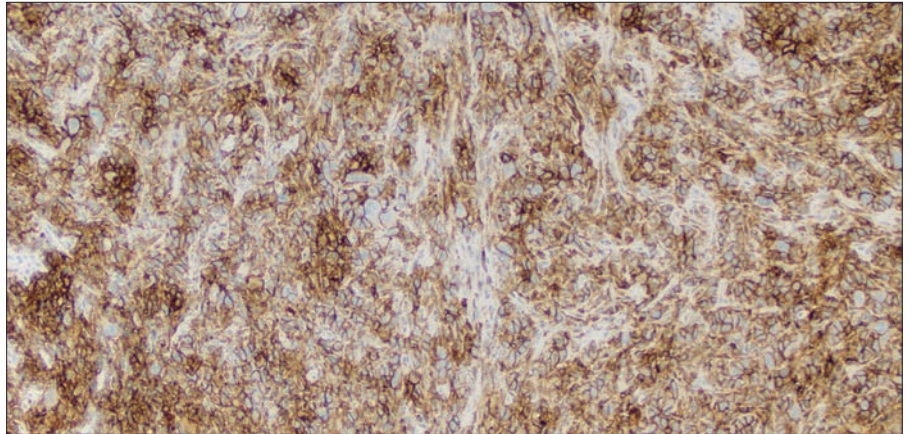


**Figure 37c:** 40x magnification

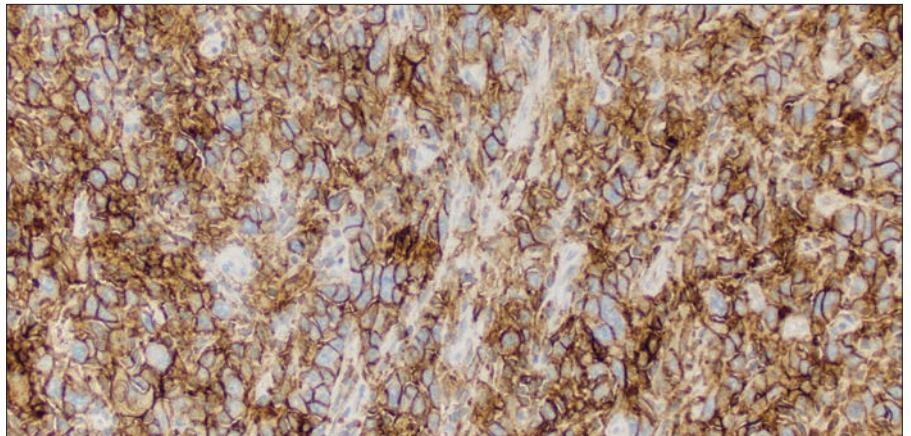
**Figure 37a–37c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS  $\geq$  50%.



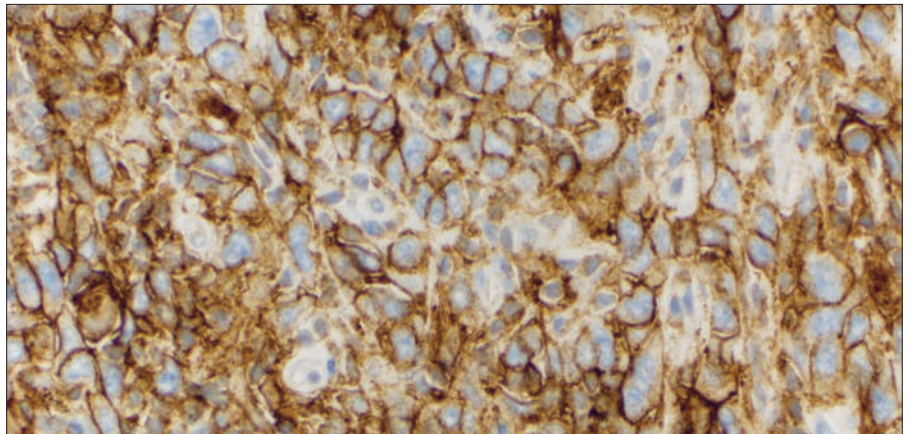
**Case 12: TPS  $\geq$  50%**



**Figure 38a:** 10x magnification



**Figure 38b:** 20x magnification

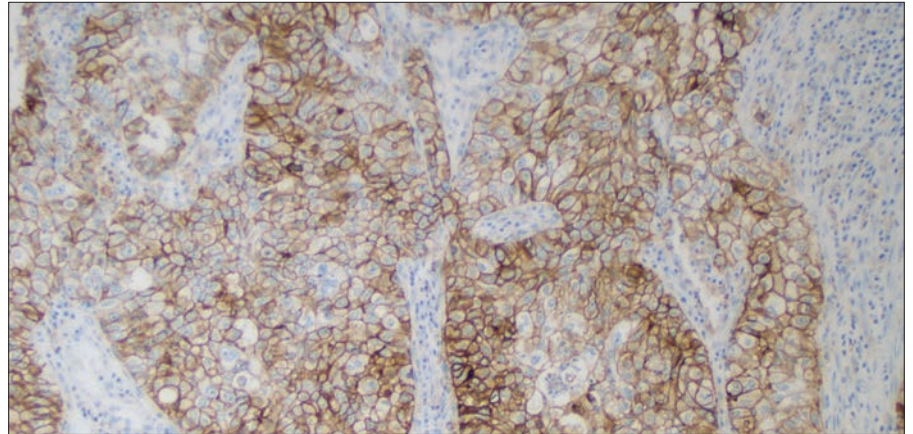


**Figure 38c:** 40x magnification

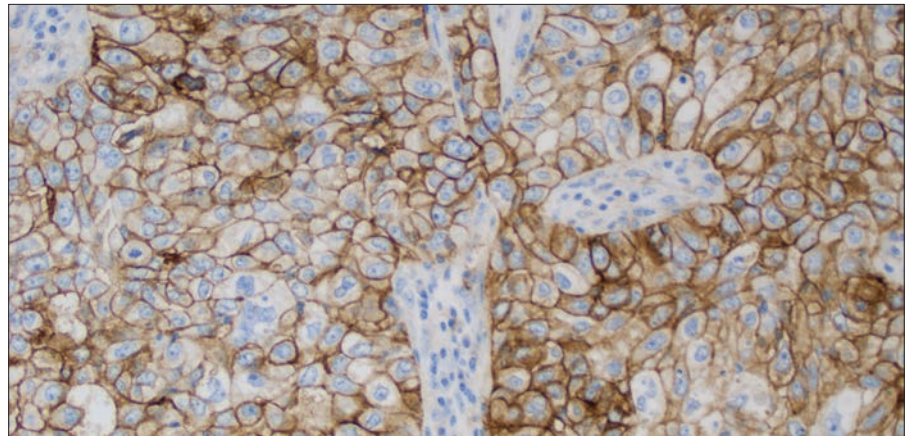
**Figure 38a–38c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS  $\geq$  50%.



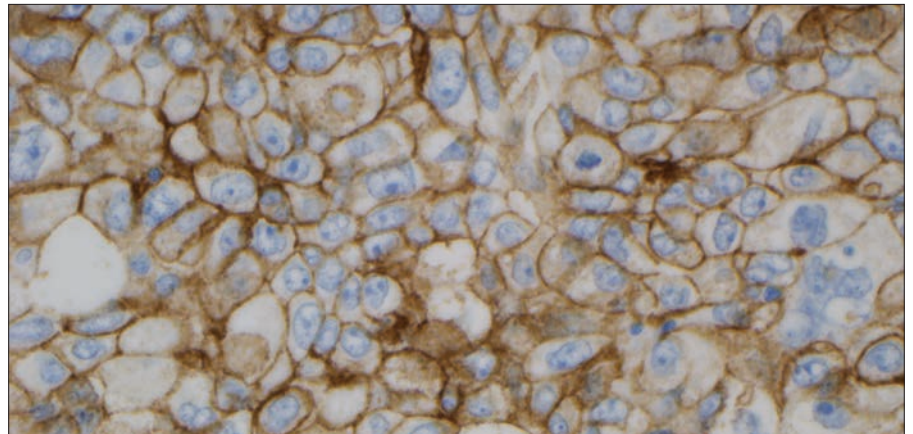
**Case 13: TPS  $\geq$  50%**



**Figure 39a:** 10x magnification



**Figure 39b:** 20x magnification

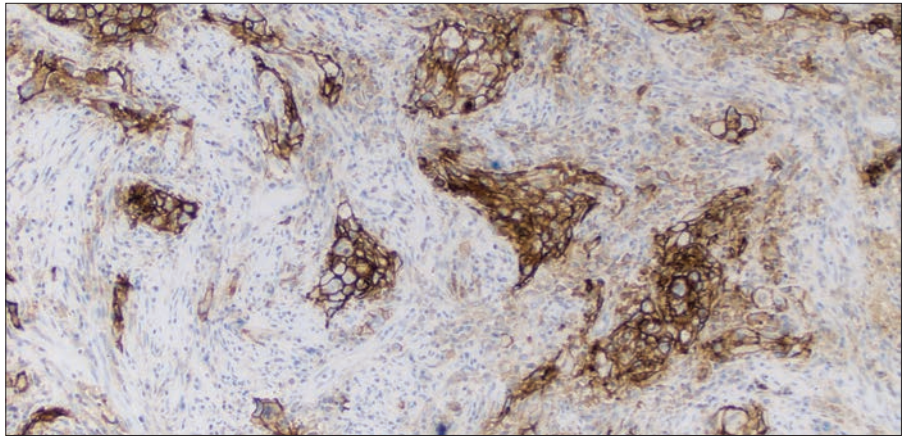


**Figure 39c:** 40x magnification

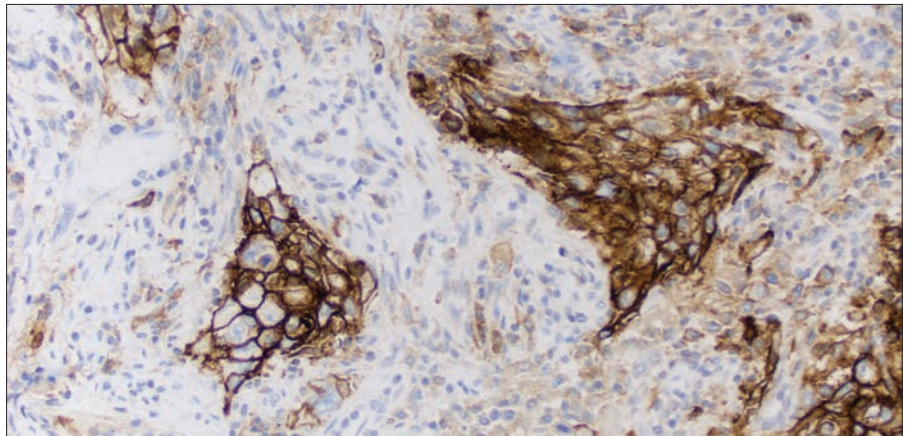
**Figure 39a–39c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS  $\geq$  50%.



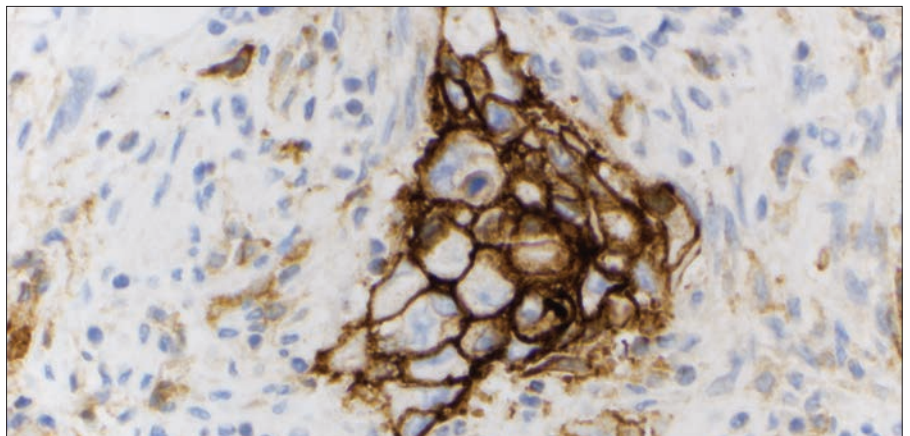
**Case 14: TPS  $\geq$  50%**



**Figure 40a:** 10x magnification



**Figure 40b:** 20x magnification

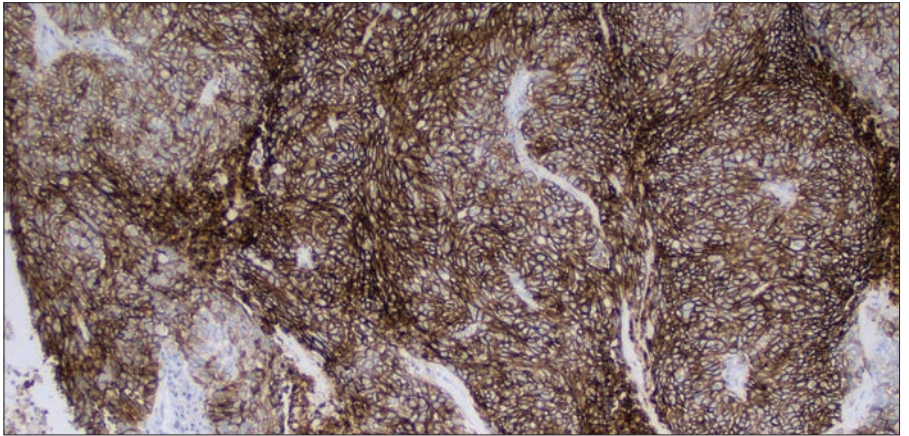


**Figure 40c:** 40x magnification

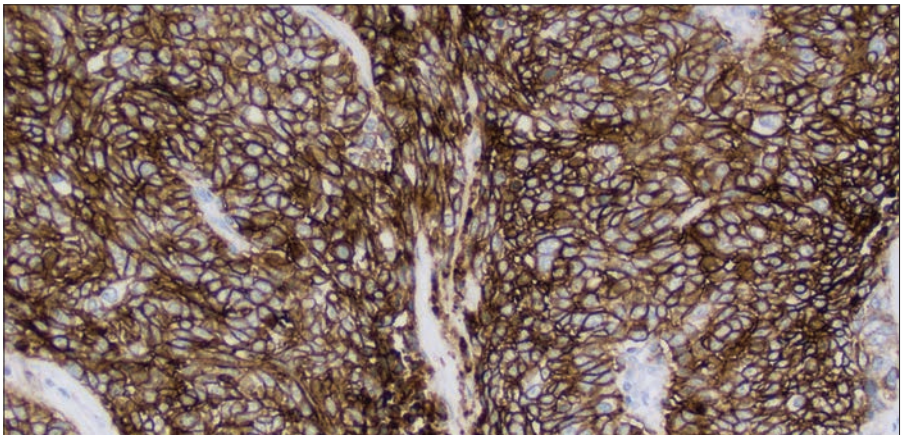
**Figure 40a–40c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS  $\geq$  50%.



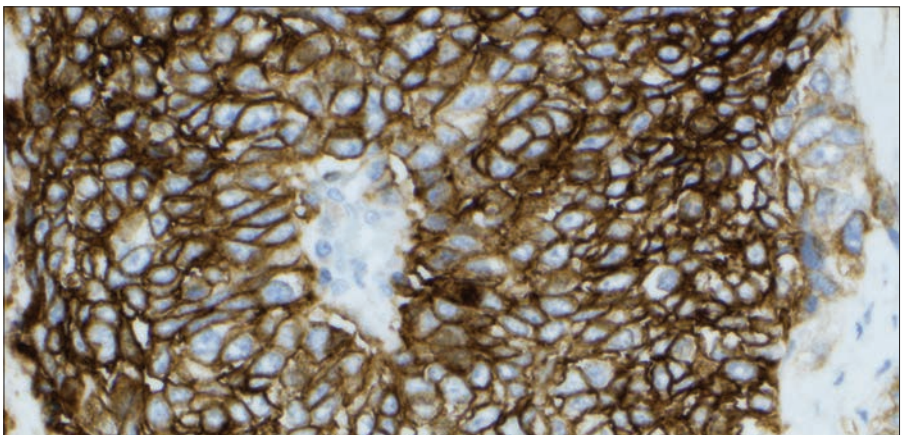
**Case 15: TPS  $\geq$  50%**



**Figure 41a:** 10x magnification



**Figure 41b:** 20x magnification



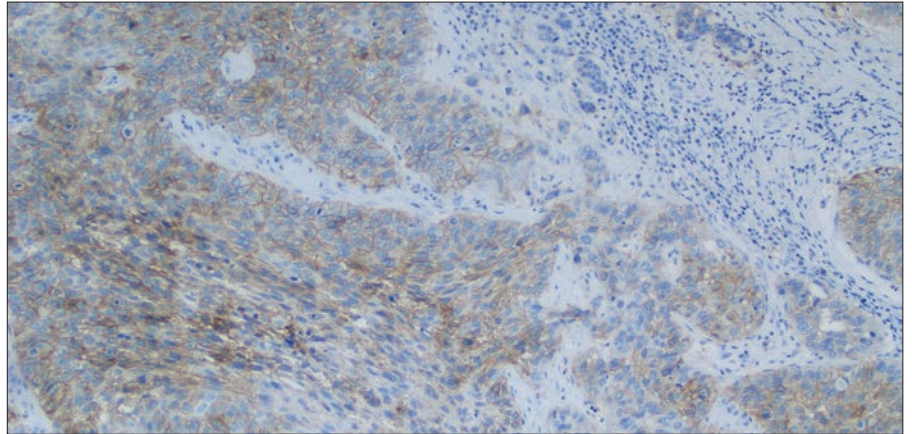
**Figure 41c:** 40x magnification

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

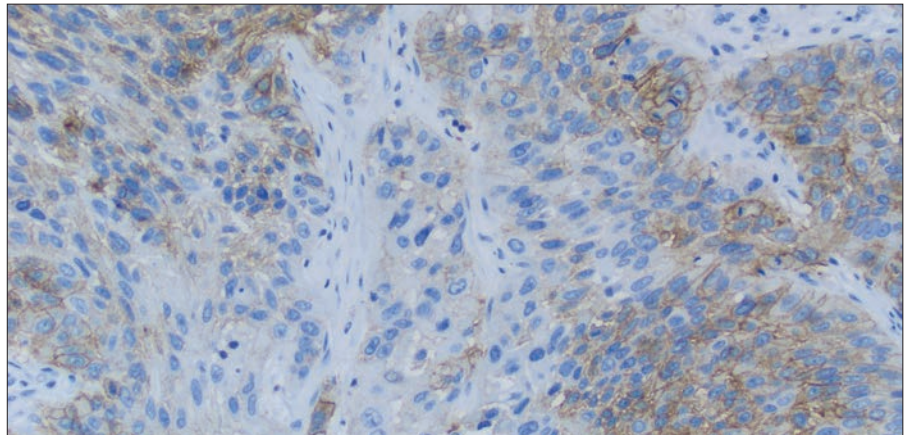


**PD-L1 IHC 22C3 pharmDx**  
**Near  $\geq 50\%$  Cut-off Case**  
**Examples (TPS 40–60%)**

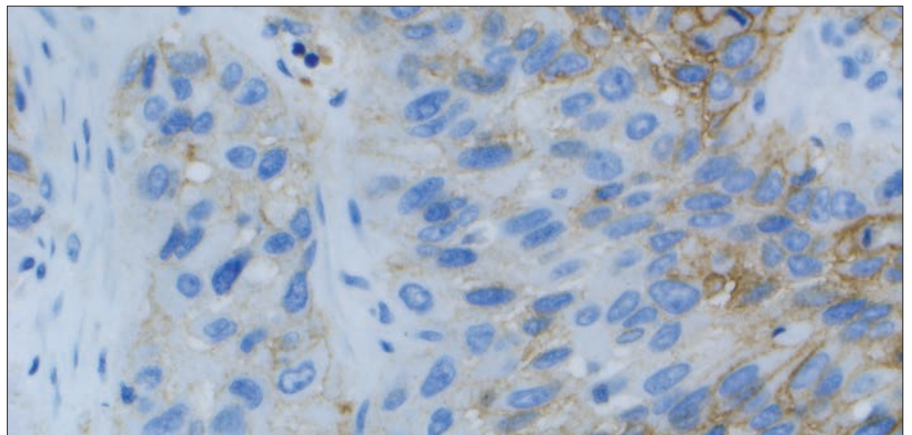
**Challenging Case 1: Near  $\geq 50\%$  Cut-off (TPS 40–60%)**



**Figure 42a:** 10x magnification



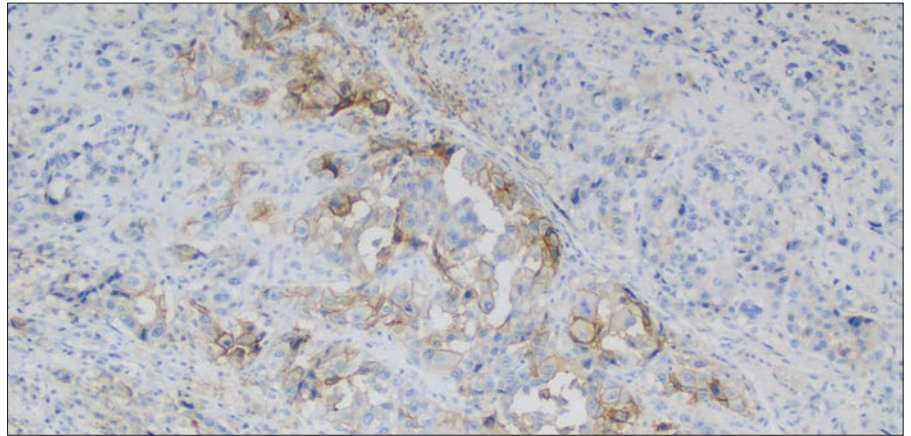
**Figure 42b:** 20x magnification



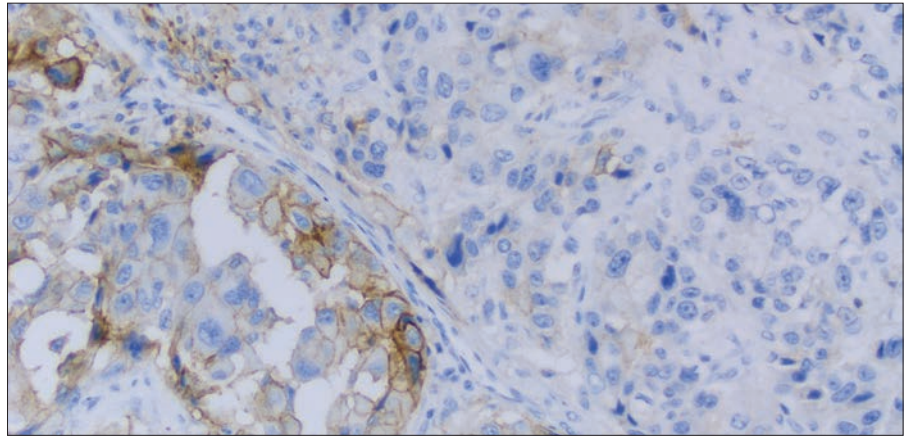
**Figure 42c:** 40x magnification

**Figure 42a–42c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS 40%.

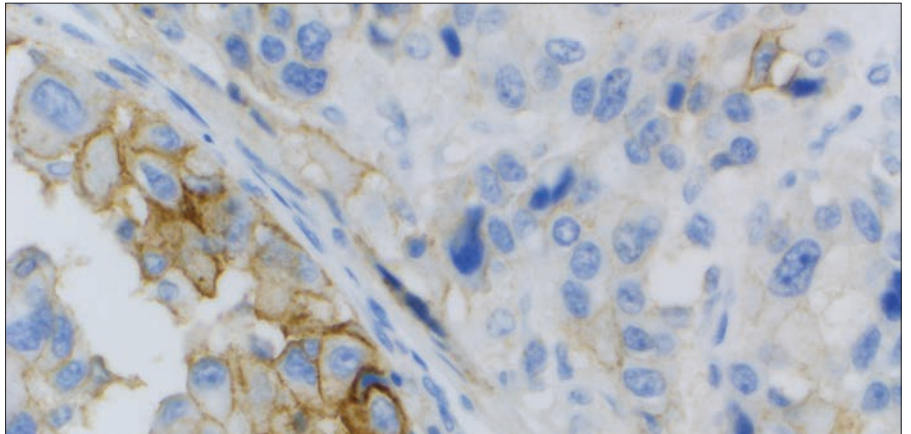
## Challenging Case 2: Near $\geq 50\%$ Cut-off (TPS 40–60%)



**Figure 43a:** 10x magnification



**Figure 43b:** 20x magnification

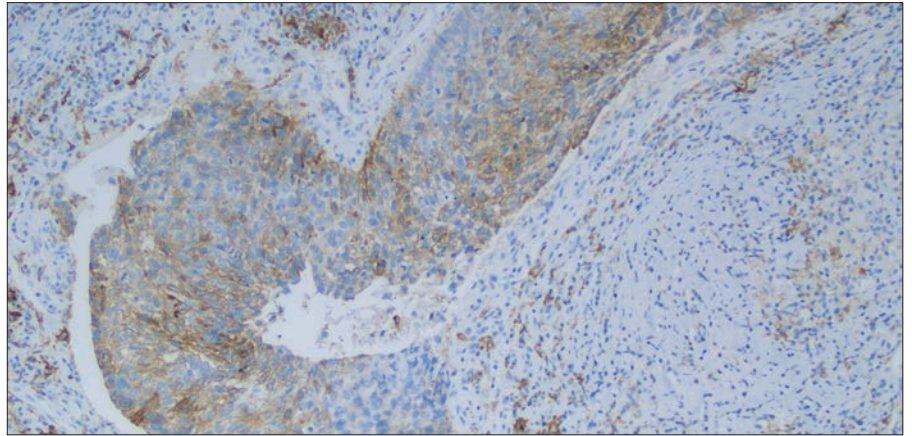


**Figure 43c:** 40x magnification

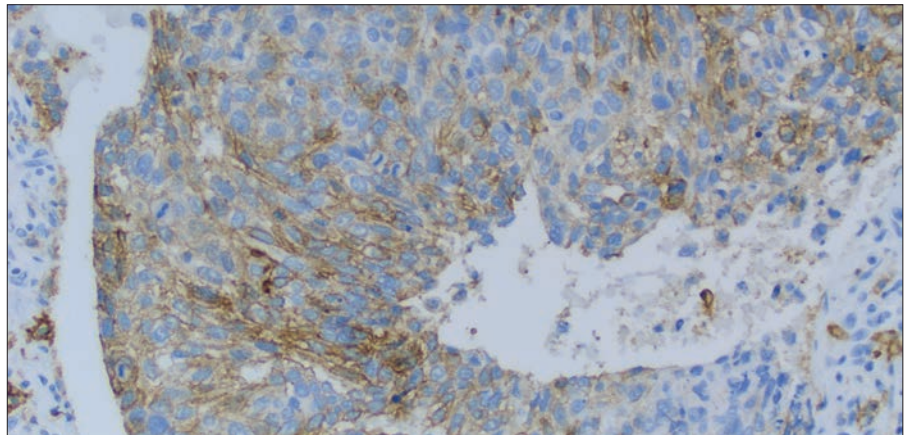
**Figure 43a–43c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS 40%.



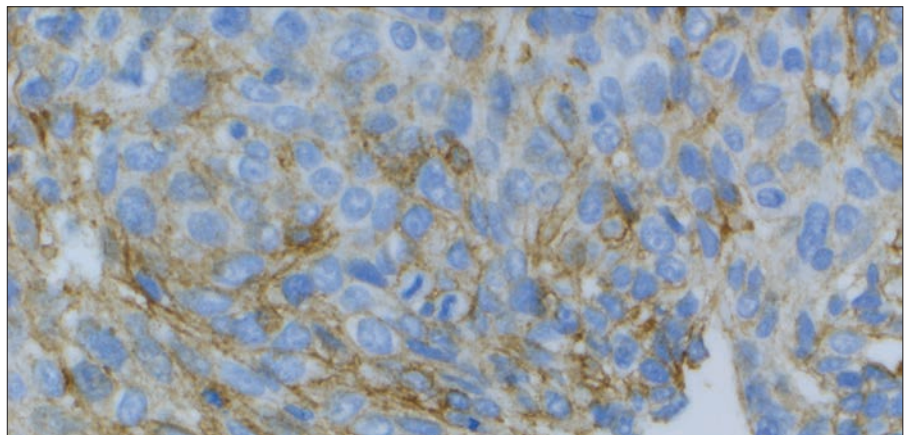
### Challenging Case 3: Near $\geq 50\%$ Cut-off (TPS 40–60%)



**Figure 44a:** 10x magnification



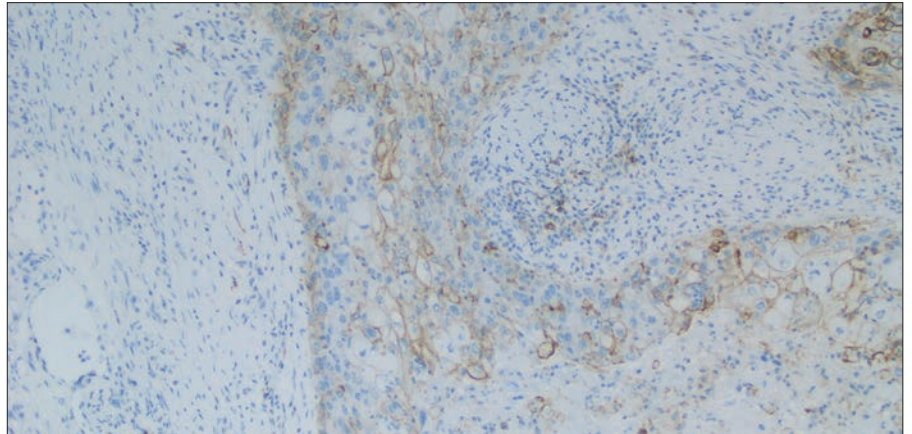
**Figure 44b:** 20x magnification



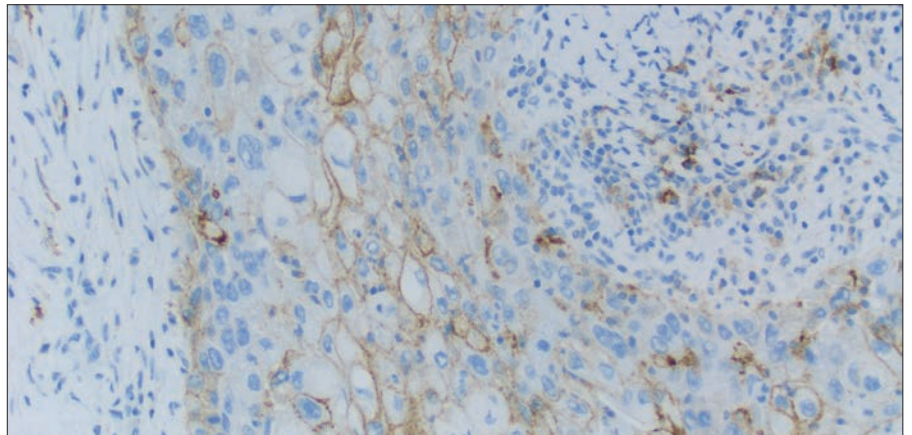
**Figure 44c:** 40x magnification

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

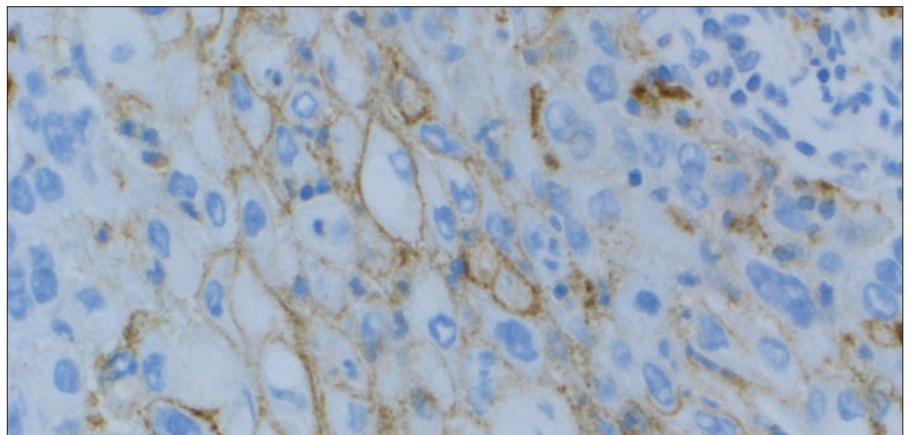
#### Challenging Case 4: Near $\geq 50\%$ Cut-off (TPS 40–60%)



**Figure 45a:** 10x magnification



**Figure 45b:** 20x magnification

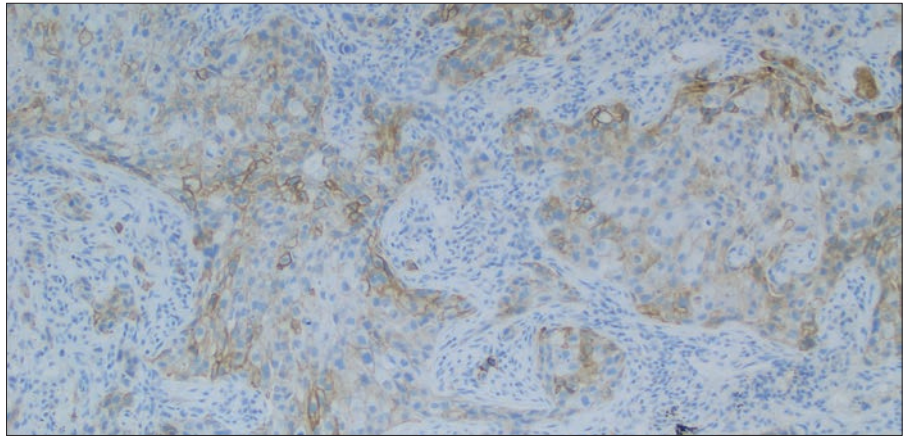


**Figure 45c:** 40x magnification

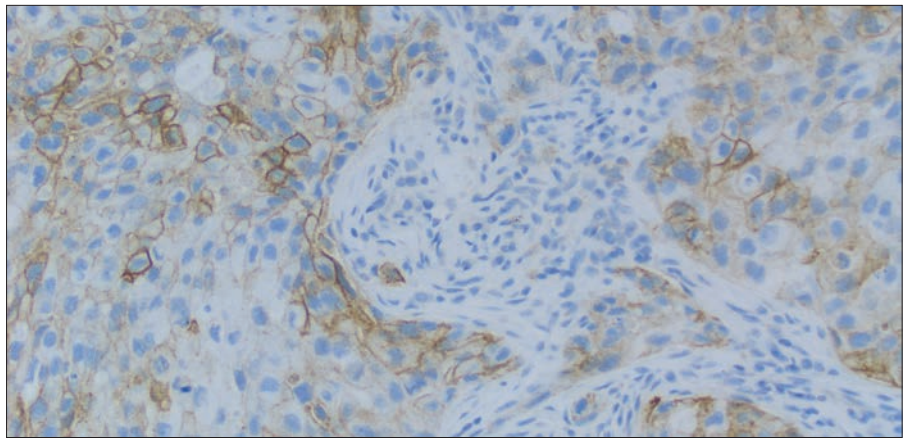
**Figure 45a–45c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS 60%.



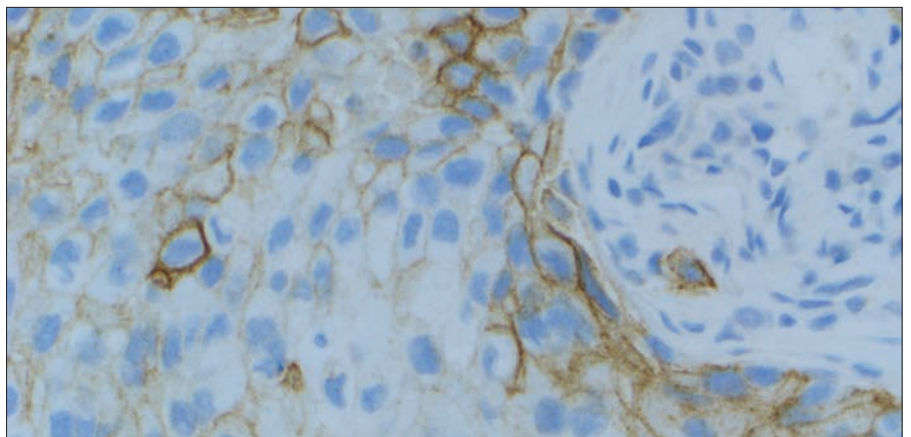
### Challenging Case 5: Near $\geq 50\%$ Cut-off (TPS 40–60%)



**Figure 46a:** 10x magnification



**Figure 46b:** 20x magnification

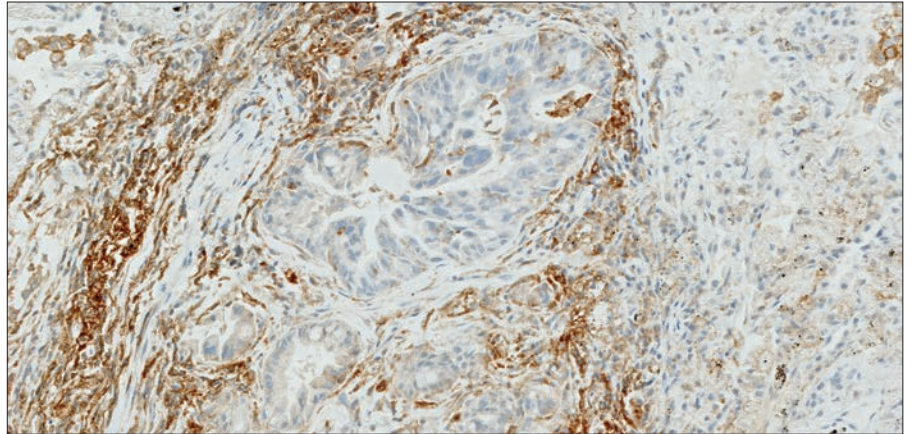


**Figure 46c:** 40x magnification

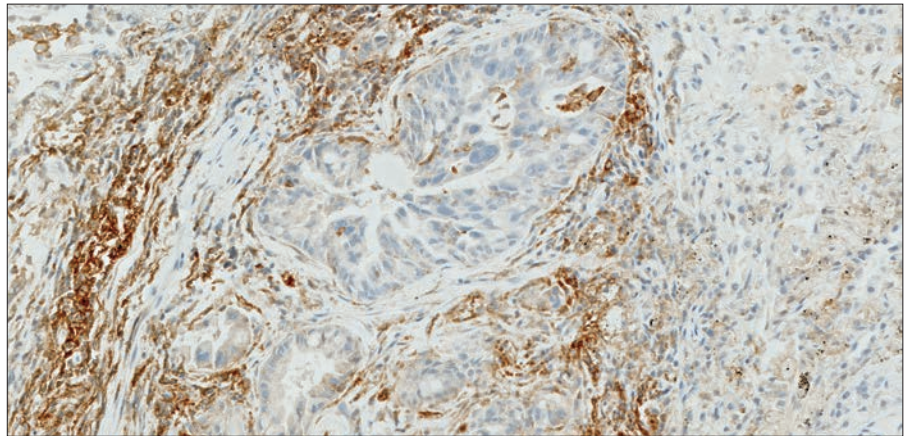
**Figure 46a–46c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS 60%.

PD-L1 IHC 22C3 pharmDx  
Near  $\geq 1\%$  Cut-off Case  
Examples (TPS 0–10%)

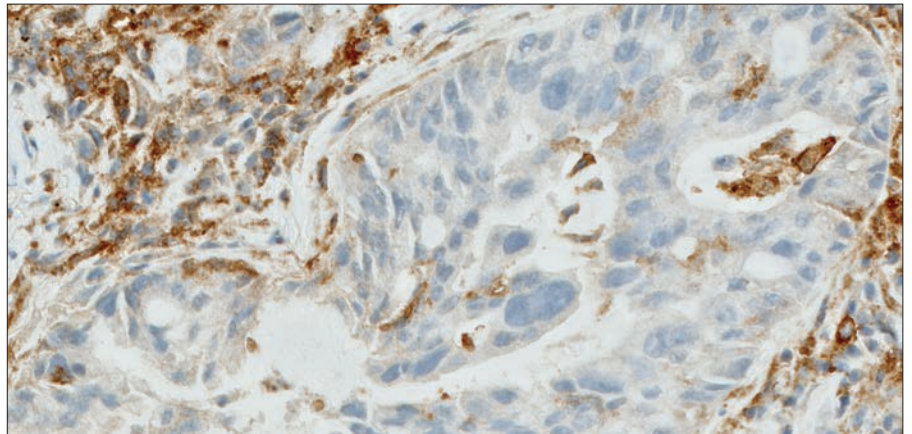
**Challenging Case 6: Near  $\geq 1\%$  Cut-off (TPS 0–10%)**



**Figure 47a:** 10x magnification



**Figure 47b:** 20x magnification

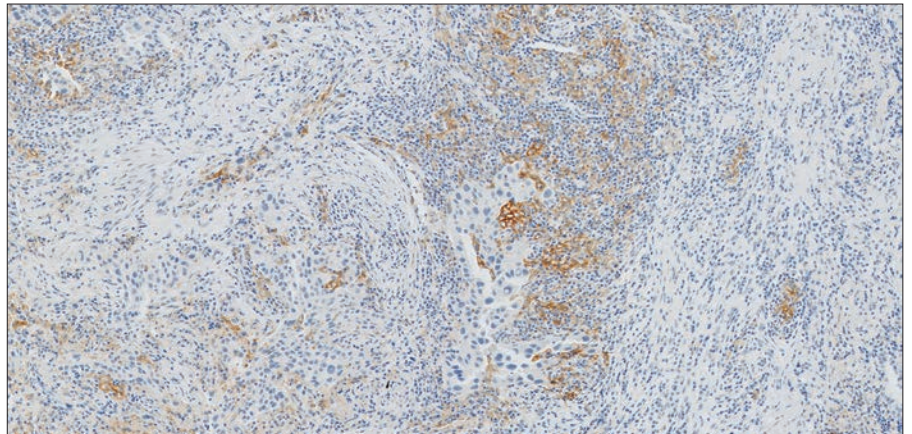


**Figure 47c:** 40x magnification

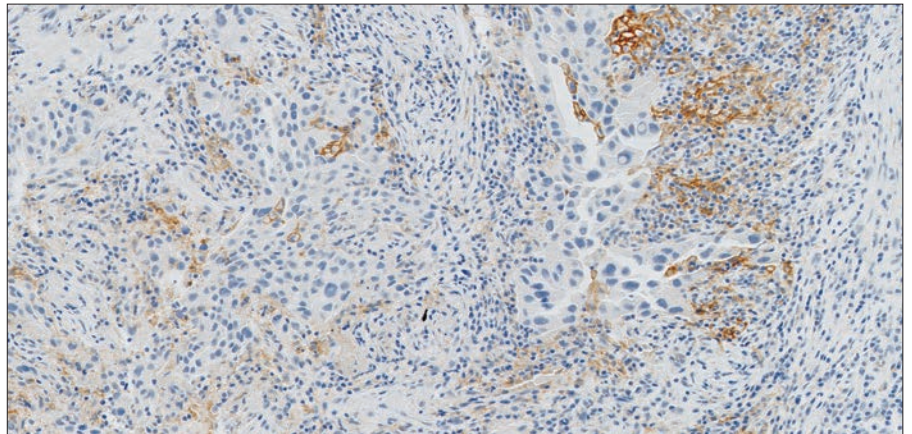
**Figure 47a–47c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS  $< 1\%$ .



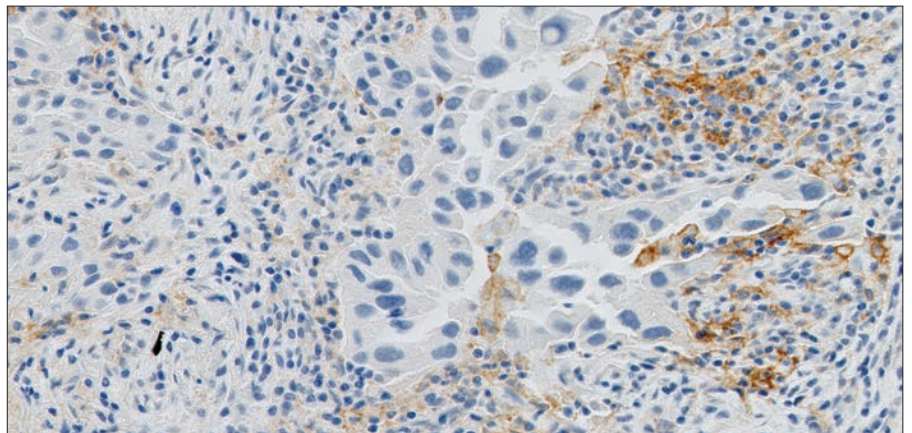
### Challenging Case 7: Near $\geq 1\%$ Cut-off (TPS 0–10%)



**Figure 48a:** 10x magnification



**Figure 48b:** 20x magnification

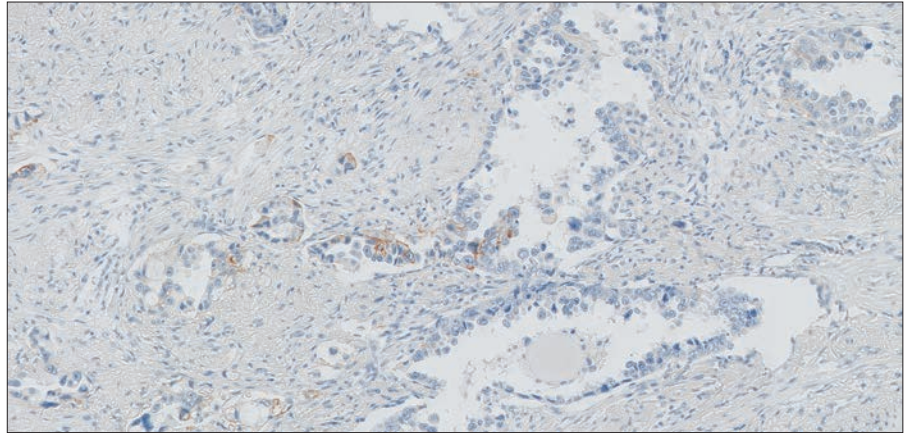


**Figure 48c:** 40x magnification

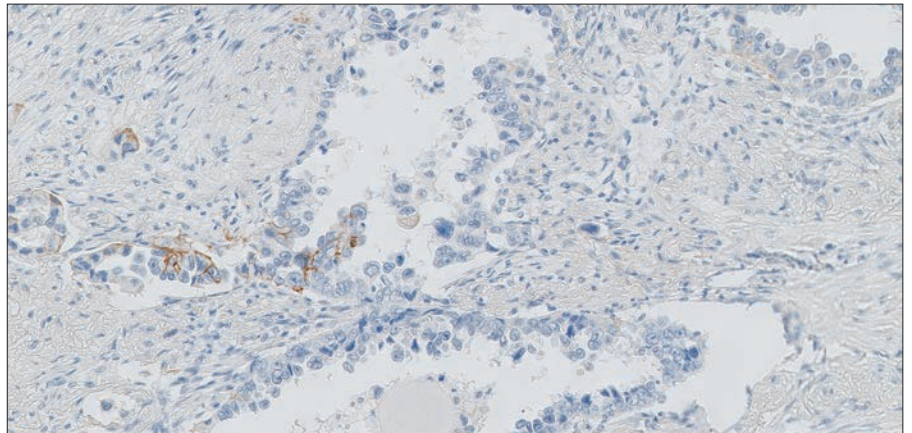
**Figure 48a–48c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS  $< 1\%$ .



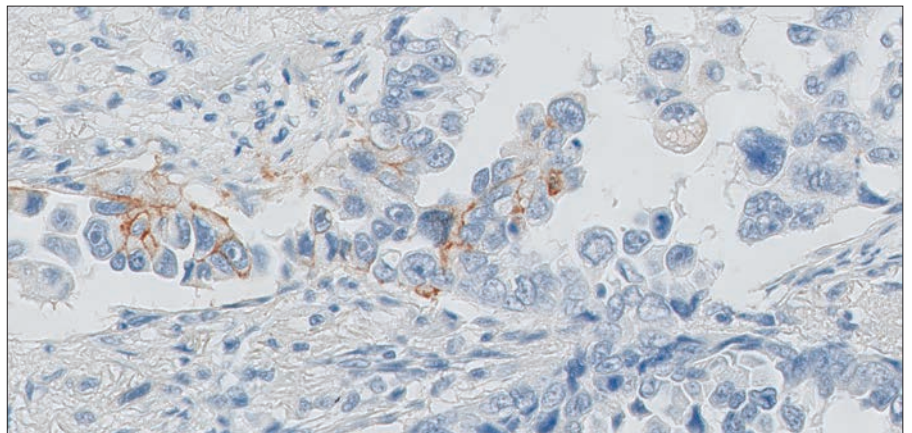
### Challenging Case 8: Near $\geq 1\%$ Cut-off (TPS 0–10%)



**Figure 49a:** 10x magnification



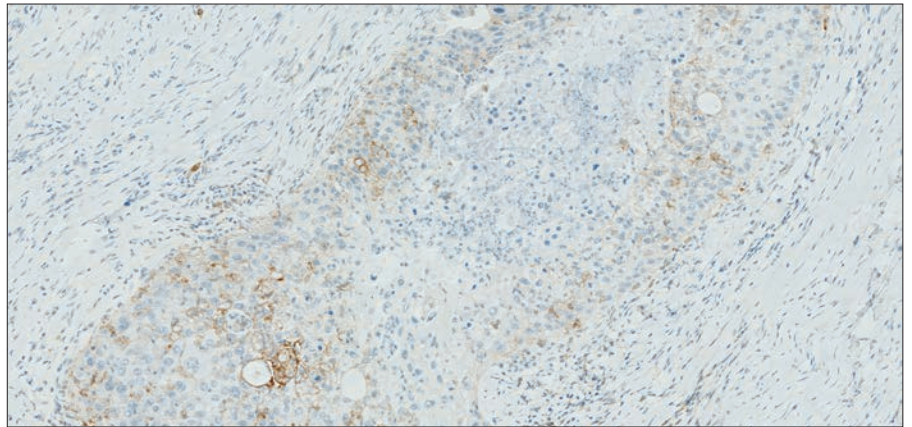
**Figure 49b:** 20x magnification



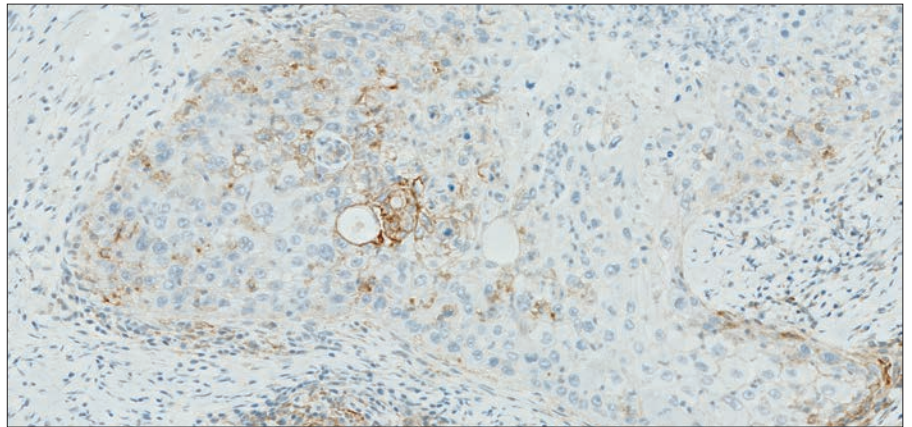
**Figure 49c:** 40x magnification

**Figure 49a–49c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS 1–10%.

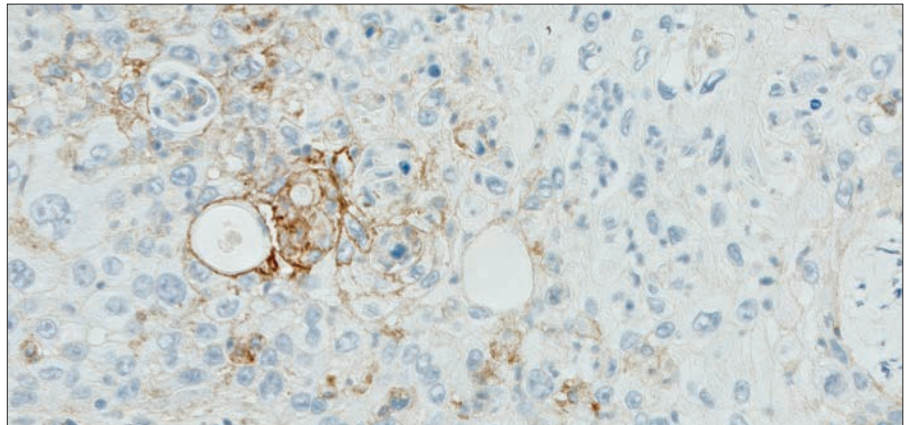
### Challenging Case 9: Near $\geq 1\%$ Cut-off (TPS 0–10%)



**Figure 50a:** 10x magnification



**Figure 50b:** 20x magnification

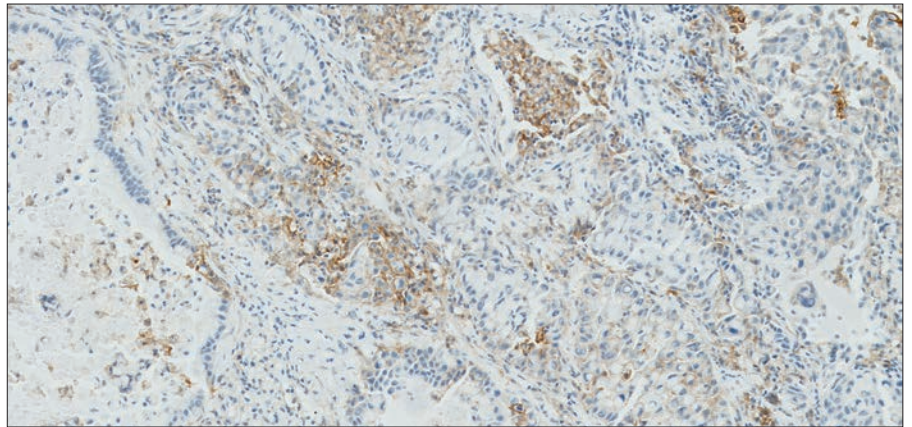


**Figure 50c:** 40x magnification

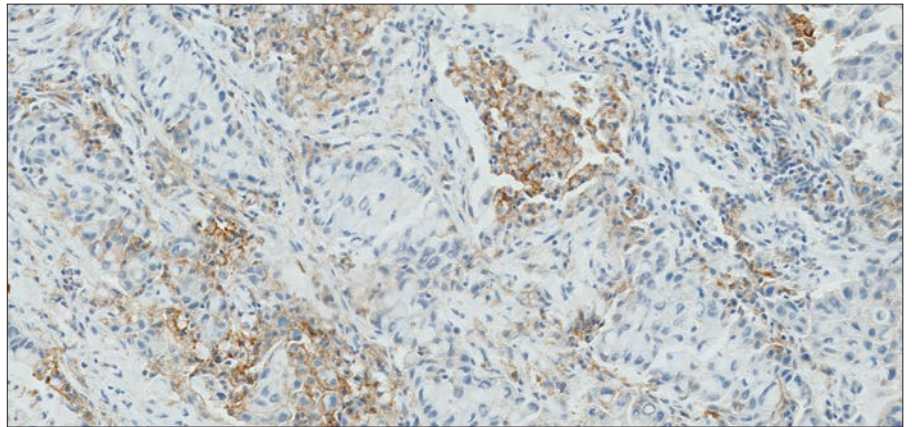
**Figure 50a–50c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS 1–10%.



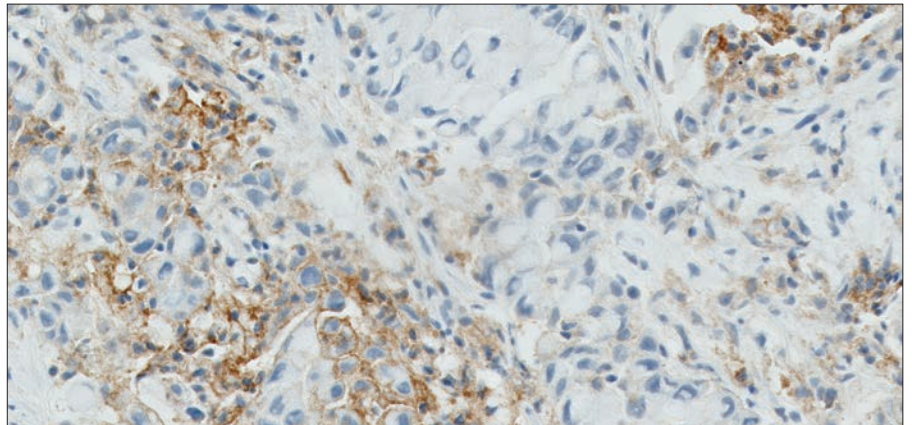
### Challenging Case 10: Near $\geq 1\%$ Cut-off (TPS 0–10%)



**Figure 51a:** 10x magnification



**Figure 51b:** 20x magnification



**Figure 51c:** 40x magnification

**Figure 51a–51c:** NSCLC specimen stained with PD-L1 antibody exhibiting TPS 1–10%.



# 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 sections, and incomplete rinsing of slides.

The use of fixatives other than neutral buffered formalin may be a source of background staining. Background staining with PD-L1 IHC 22C3 pharmDx is rare.

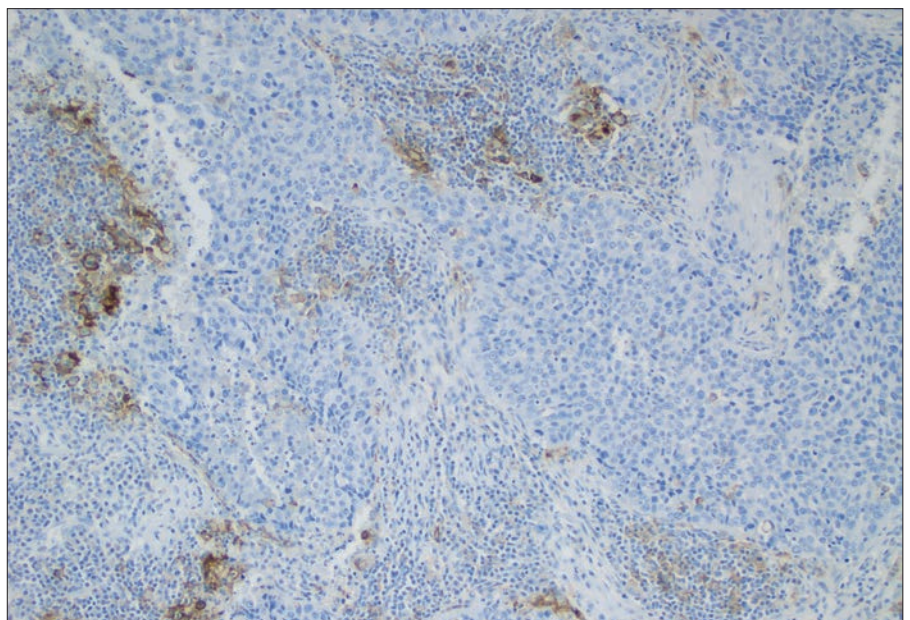
### Possible Causes of Background

- Improper 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

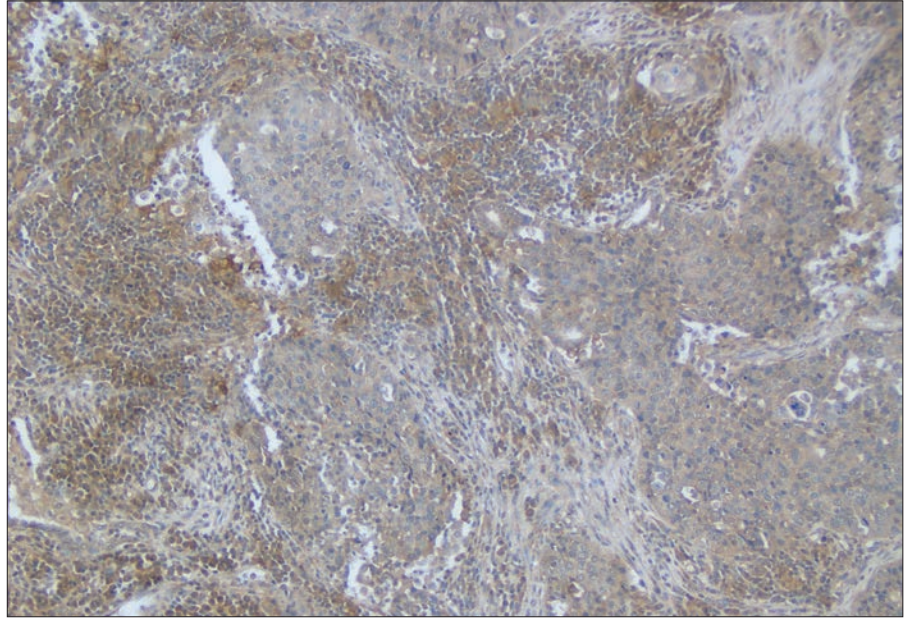
The non-specific background staining of the negative test specimen is useful in determining the level of background staining in the positive test specimen.

### Key point

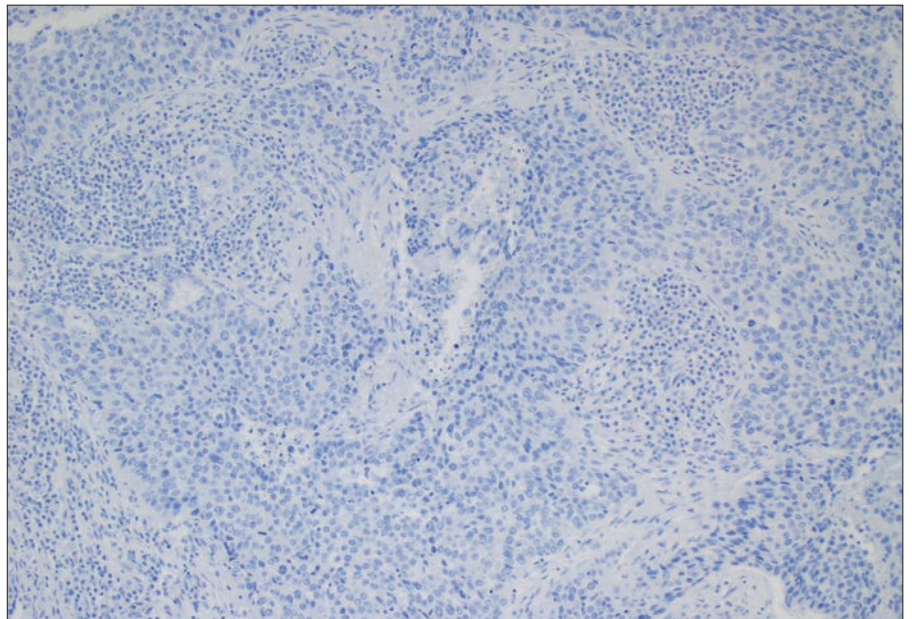
All specimens must have  $\leq 1+$  non-specific background staining



**Figure 52:** NSCLC stained with PD-L1 primary antibody exhibiting acceptable non-specific background staining (20x magnification).



**Figure 53:** NSCLC stained with PD-L1 primary antibody exhibiting unacceptable non-specific background staining (> 1+) (20x magnification).



**Figure 54:** NSCLC stained with Negative Control Reagent (NCR) exhibiting acceptable non-specific background staining (20x magnification).



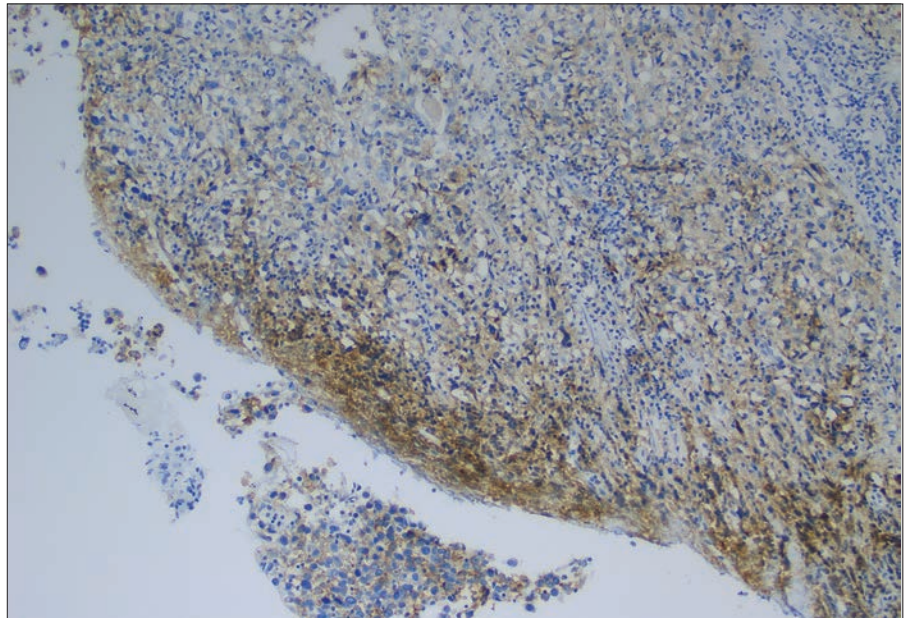
## Edge Artifact

Commonly, edge artifacts are linked to the pre-analytic handling of the tissue.

- Inadequate processing of thick tissue samples may mimic edge artifact by rendering the central portion of the tissue sub-optimally fixed relative to the peripheral areas. In these circumstances, the immunoreactivity based on the sub-optimal central portion may be mistakenly interpreted as false-negative as optimal fixation is only present at the periphery
- Frequently, increased staining is observed around the periphery of the tissue specimen, known as the “edge artifact”
- Edge artifacts can be due to drying of the tissue specimen prior to fixation or during the staining procedure
- If the positive reaction is only at the edge of the tissue section (i.e., a few cell layers of staining at the periphery and ending abruptly with penetration into the centrally located tumor), scoring at the edge of the tissue specimen should be avoided

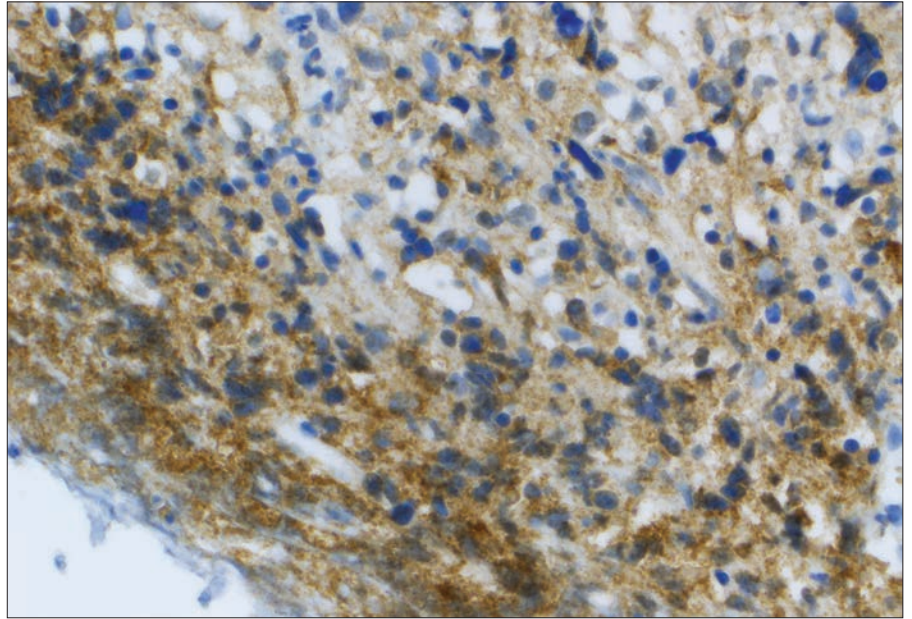
### Key point

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



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





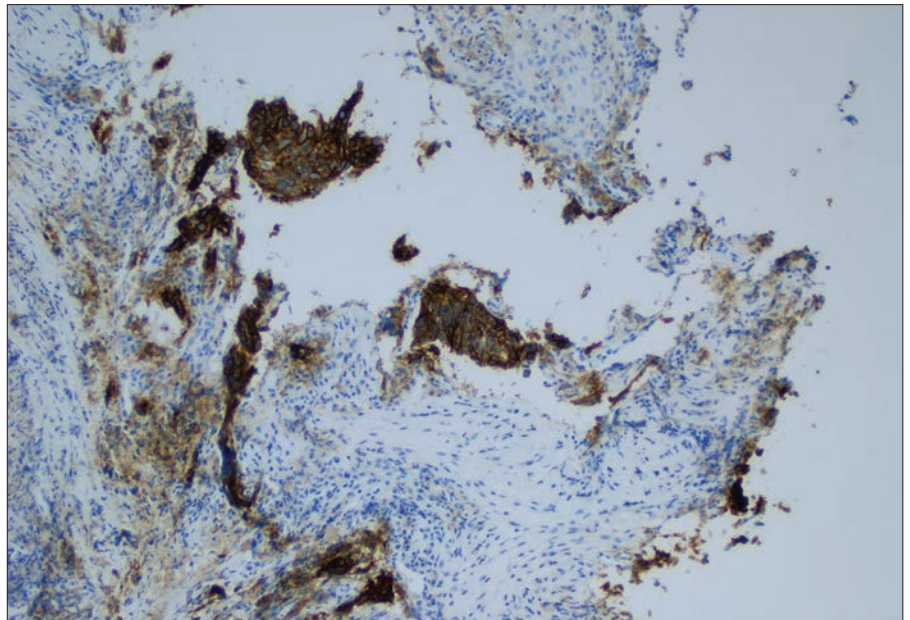
**Figure 55b:** NSCLC stained with PD-L1 primary antibody exhibiting edge artifact staining; edge staining should be excluded from the scoring (20x magnification).

## Crush Artifact

- Crush artifact is closely related to edge artifact. This artifact may be encountered more often in transbronchial biopsies. The compression of the tissues along the edges of the specimen can produce a linear staining that has to be interpreted as an artifact.
- Inadvertent crushing of the tissue occasionally occurs during sectioning, resulting in morphologically distorted cellular architecture
- When compared to surrounding cells, stronger staining may be observed in crushed cells. Crushed cells typically demonstrate condensed nuclei. Crushed cells should be avoided in scoring

### Key point

Scoring of crush artifact should be avoided if staining is inconsistent with entire specimen



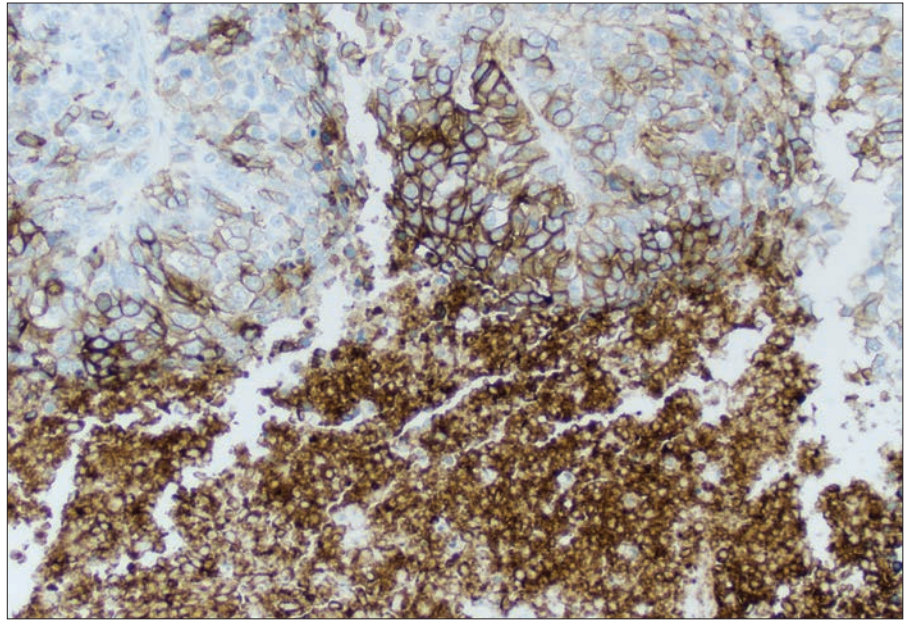
**Figure 56:** NSCLC stained with PD-L1 primary antibody exhibiting crush artifact (10x magnification).

## Necrosis

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

### Key point

Scoring of necrotic areas should be excluded from the TPS



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

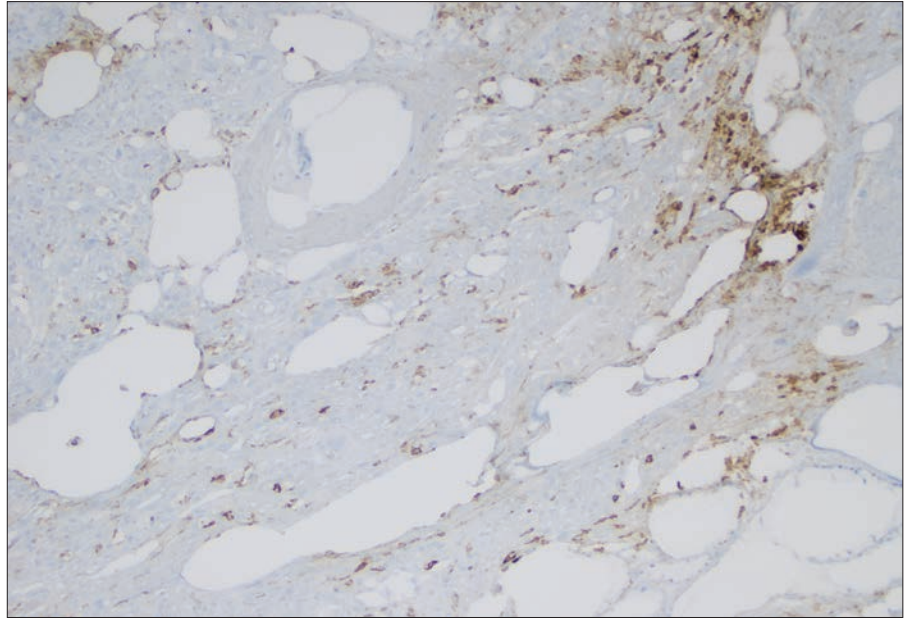


## Poor Fixation

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

### Key point

Proper fixation is important for accurate diagnosis



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

# Troubleshooting Guide

## Troubleshooting Guidelines for PD-L1 IHC 22C3 pharmDx

For further troubleshooting help, contact your local Agilent representative.

Problem	Probable Cause	Suggested Action
No staining of slides	Programming error	Verify that the PD-L1 IHC 22C3 pharmDx program was selected for programming of slides
	Lack of reaction with DAB+ Substrate-Chromogen Solution (DAB)	Verify that DAB+ Substrate-Chromogen Solution was prepared properly
	Sodium azide in wash buffer	Use only Dako Wash Buffer (Code K8007)
	Degradation of Control Slide	Check kit expiration date and kit storage conditions on outside of package
Weak staining of specimen slides	Inappropriate fixation method used	Ensure that only approved fixatives and fixation methods are used
Weak staining of specimen slides or of the positive cell line on the Agilent-provided Control Cell Line Slide	Inadequate target retrieval	Verify that the 3-in-1 pre-treatment procedure was correctly performed
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
	Non-specific binding of reagents to tissue section	Check for proper fixation of the specimen and/or the presence of necrosis
Tissue detached from slides	Use of incorrect microscope slides	Use Dako FLEX IHC Microscope Slides, (Code K8020), or Fisherbrand Superfrost Plus slide
Excessively strong specific staining	Inappropriate fixation method used	Ensure that only approved fixatives and fixation methods are used
	Inappropriate wash buffer used	Use only Dako Wash Buffer, Code K8007
Target Retrieval Solution is cloudy in appearance when heated	When heated, the Target Retrieval Solution turns cloudy in appearance	This is normal and does not influence staining

# Clinical Performance Evaluation

## Trial 24: Controlled trial of first-line treatment of patients with NSCLC

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

**Table 4: Efficacy Results in Trial 24**

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

\* Assessed by BICR using RECIST 1.1

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

‡ Based on stratified Log rank test

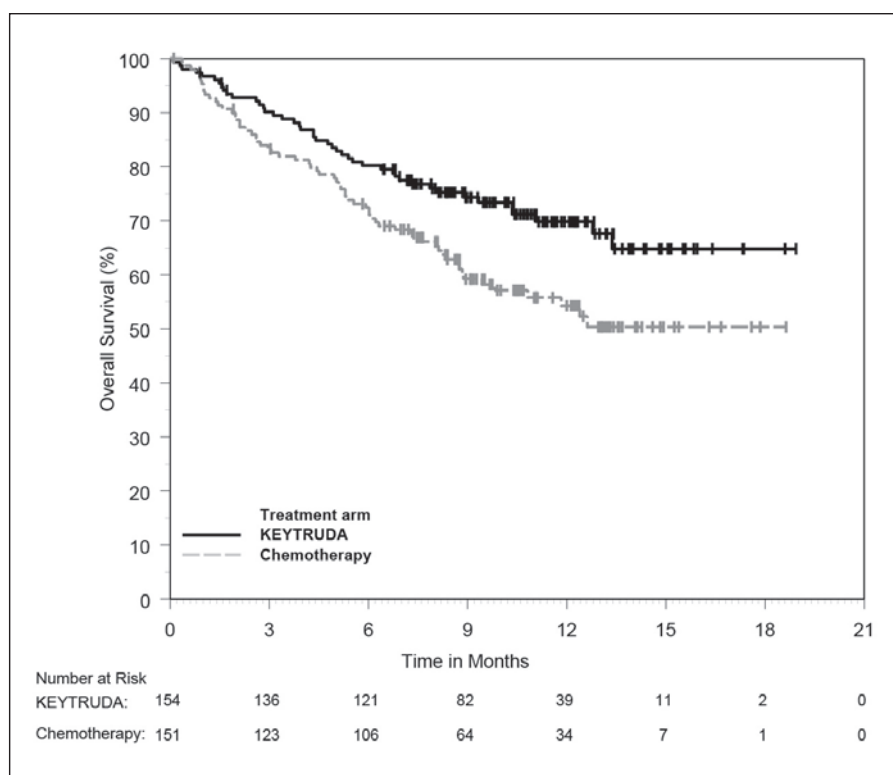
NA = not available



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

The major efficacy outcome measure was progression-free survival (PFS) as assessed by blinded independent central review (BICR) using Response Evaluation Criteria on Solid Tumors Version 1.1 (RECIST 1.1). Additional efficacy outcome measures were overall survival (OS) and objective response rate (ORR) as assessed by BICR using RECIST 1.1. Table 4 summarizes key efficacy measures for the entire intent to treat (ITT) population.

Among the 69 patients randomized to KEYTRUDA 200 mg with an objective response, response durations ranged from 1.9+ to 14.5+ months. Eighty-eight percent of these responders had a response duration of 6 months or longer (based on Kaplan-Meier estimation; Figure 59).



**Figure 59:** Kaplan-Meier Curve for Overall Survival in Trial 24.

### Trial 10: Controlled trial of NSCLC patients previously treated with chemotherapy

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

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

The level of agreement achieved between the CTA and PD-L1 IHC 22C3 pharmDx is shown in Table 5.

**Table 5:** CTA vs. PD-L1 IHC 22C3 pharmDx Agreement

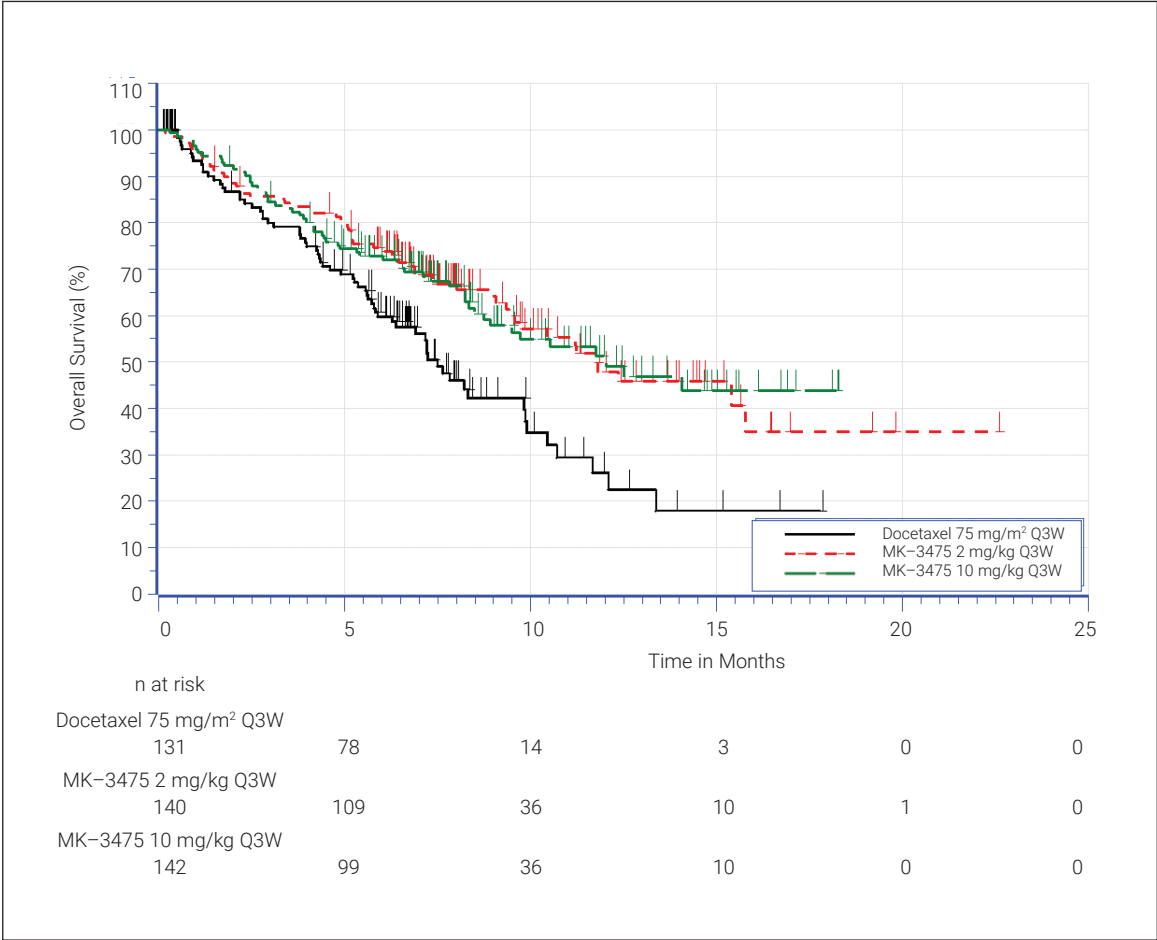
Agreement Rates	PD-L1 Cut-off	Negative Percent Agreement (95% Confidence Interval (CI))	Positive Percent Agreement (95% Confidence Interval (CI))
CTA vs. PD-L1 IHC 22C3 pharmDx	TPS $\geq 1\%$	94.5% [91.4%–96.6%]	80.0% [76.9%–82.8%]
	TPS $\geq 50\%$	98.3% [97.1%–99.0%]	73.2% [67.9%–77.9%]

Among randomized patients having PD-L1 expression by PD-L1 IHC 22C3 pharmDx, the demographic and other baseline characteristics were well balanced between the treatment arms. The median age was 63 years (44% age 65 or older). The majority of patients were white (77%) and male (58%); baseline ECOG performance status was 0 (29%) or 1 (71%). Seventy-eight percent (78%) of patients were former/current smokers. Twenty-two percent (22%) of patients had squamous histology and 69% had non-squamous histology. The baseline and demographic characteristics were similarly well balanced across pembrolizumab and docetaxel arms in the overall clinical study.

Efficacy results are summarized in Tables 6 and 7 (on page 73). KEYTRUDA demonstrated durable clinical benefit in NSCLC patients with PD-L1 expression (TPS  $\geq 1\%$ ), which was enhanced in patients with high PD-L1 expression (TPS  $\geq 50\%$ ), as determined by PD-L1 IHC 22C3 pharmDx. The magnitude of benefit was comparable to that in the overall clinical trial. The tables on page 73 summarize key efficacy measures in the overall population with PD-L1 expression (TPS  $\geq 1\%$ ) and in the high PD-L1 expression (TPS  $\geq 50\%$ ) subset for the overall clinical study (TPS  $\geq 1\%$  by CTA) and in the population with PD-L1 expression by PD-L1 IHC 22C3 pharmDx. The Kaplan-Meier curve for OS (TPS  $\geq 1\%$ ), as determined by PD-L1 IHC 22C3 pharmDx is shown in Figure 60. Efficacy results were similar for the 2 mg/kg and 10 mg/kg KEYTRUDA arms.

Additional robustness analyses were conducted to consider the potential impact of missing data arising from patients with PD-L1 expression (TPS  $\geq 1\%$ ) 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  $\geq 1\%$  and TPS  $\geq 50\%$  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.





**Figure 60:** Kaplan-Meier Curve for Overall Survival by Treatment Arm (TPS ≥ 1% by PD-L1 IHC 22C3 pharmDx, Intent to Treat Population).

**Table 6:** Response to KEYTRUDA in Previously Treated NSCLC Patients: Overall Clinical Study and PD-L1 IHC 22C3 pharmDx Positive Patients: PD-L1 TPS  $\geq$  1%

Endpoint	KEYTRUDA 2 mg/kg every 3 weeks		KEYTRUDA 10 mg/kg every 3 weeks		Docetaxel 75 mg/m <sup>2</sup> every 3 weeks	
	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx
<b>Number of Patients</b>	344	140	346	142	343	131
<b>OS</b>						
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 <sup>†</sup>	<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)
<b>PFS<sup>‡</sup></b>						
Events (%)	266 (77%)	97 (63%)	255 (74%)	103 (73%)	257 (75%)	94 (72%)
Hazard ratio* (95% CI)	0.88 (0.73, 1.04)	0.68 (0.50, 0.92)	0.79 (0.66, 0.94)	0.79 (0.59, 1.06)	---	---
p-Value <sup>†</sup>	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)
<b>Overall Response Rate<sup>‡</sup></b>						
ORR % <sup>§</sup> (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; <sup>†</sup> Based on stratified Log rank test;<sup>‡</sup> Assessed by BICR using RECIST 1.1; <sup>§</sup> All responses were partial responses**Table 7:** Response to KEYTRUDA in Previously Treated NSCLC Patients: Overall Clinical Study and PD-L1 IHC 22C3 pharmDx Positive Patients: PD-L1 TPS  $\geq$  50%

Endpoint	KEYTRUDA 2 mg/kg every 3 weeks		KEYTRUDA 10 mg/kg every 3 weeks		Docetaxel 75 mg/m <sup>2</sup> every 3 weeks	
	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx	Clinical Trial	PD-L1 IHC 22C3 pharmDx
<b>Number of Patients</b>	139	56	151	60	152	47
<b>OS</b>						
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 <sup>†</sup>	<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)
<b>PFS<sup>‡</sup></b>						
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 <sup>†</sup>	<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)
<b>Overall Response Rate<sup>‡</sup></b>						
ORR % <sup>§</sup> (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; <sup>†</sup> Based on stratified Log rank test;<sup>‡</sup> Assessed by BICR using RECIST 1.1; <sup>§</sup> All responses were partial responses

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## Notes

This image shows a single sheet of white paper with horizontal ruling lines. The lines are evenly spaced and run across the width of the page. There are no margins, text, or other markings on the paper.

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

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