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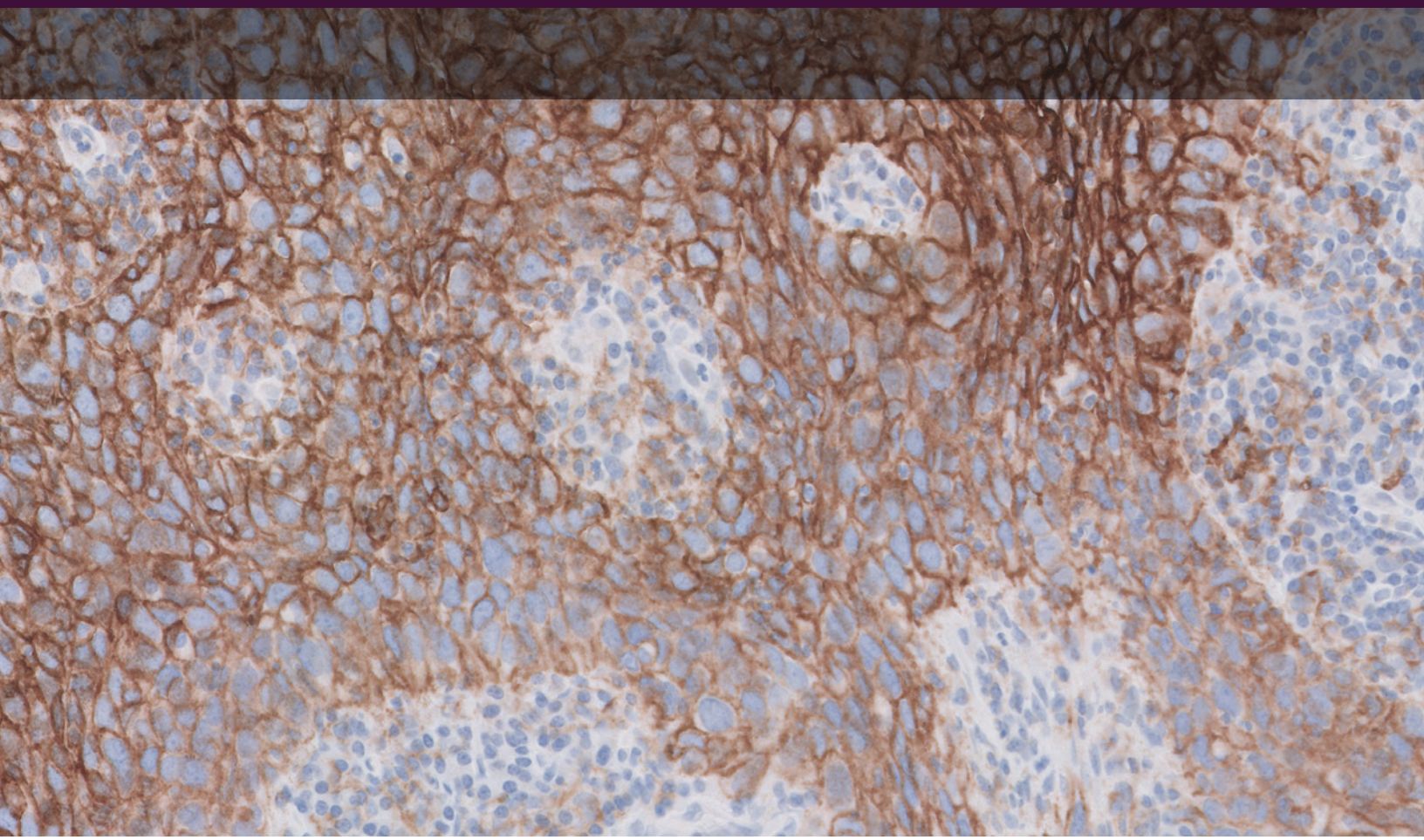
PD-L1 IHC 22C3 pharmDx Interpretation Manual – Cervical Cancer

CE-IVD–marked for in vitro diagnostic use



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

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Intended Use

For in vitro diagnostic use.

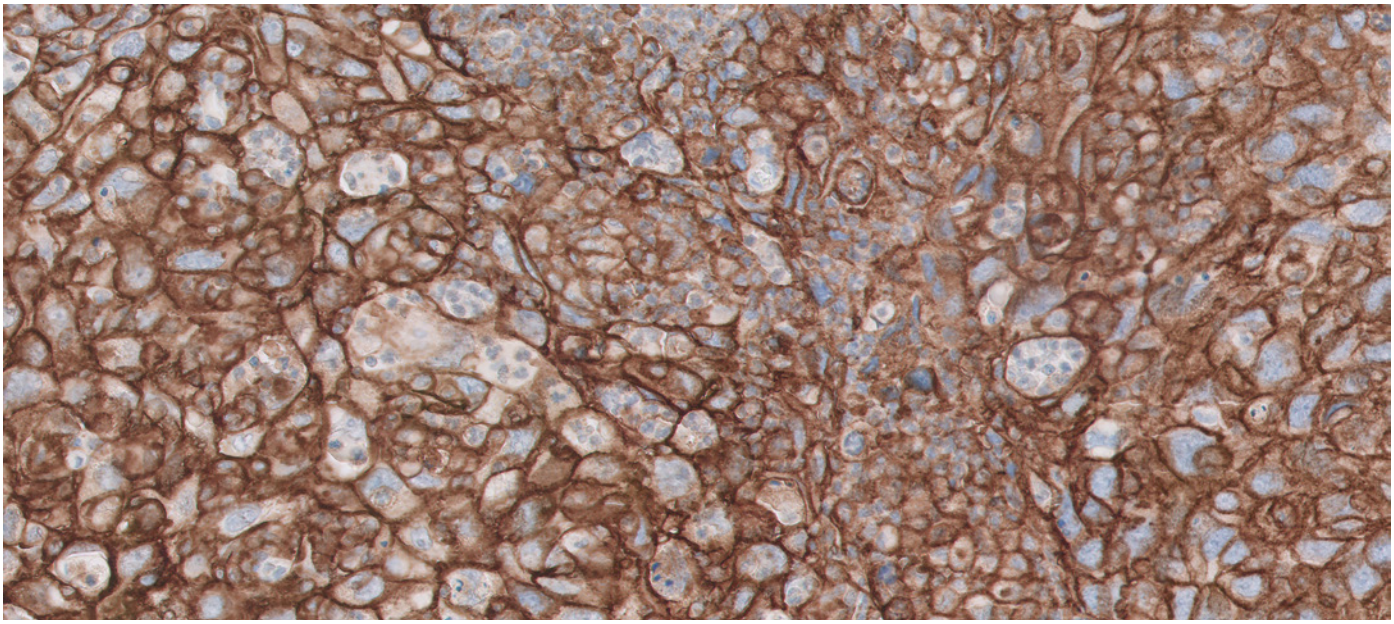
PD-L1 IHC 22C3 pharmDx is a qualitative immunohistochemical assay using monoclonal mouse anti-PD-L1, Clone 22C3, intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) cervical cancer tissue using EnVision FLEX visualization system on Autostainer Link 48.

Cervical Cancer

PD-L1 protein expression in cervical cancer 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 IHC 22C3 pharmDx is indicated as an aid in identifying cervical cancer patients for treatment with KEYTRUDA® (pembrolizumab).

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



Introduction

PD-L1 IHC 22C3 pharmDx is the only clinical trial-proven companion diagnostic CE-IVD–marked as an aid in identifying patients with cervical cancer for treatment with KEYTRUDA® (pembrolizumab).[2] 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 FFPE cervical cancer specimens. PD-L1 expression evaluation may be used to identify patients for treatment with KEYTRUDA.

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 cervical cancer 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 cervical cancer specimens stained with PD-L1 IHC 22C3 pharmDx can help individual laboratories achieve reproducible and reliable results.

PD-L1 IHC 22C3 pharmDx is considered a qualitative immunohistochemical assay. PD-L1 expression in cervical cancer 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.

Cervical cancer tissue specimens that are tested for PD-L1 expression are scored and divided into PD-L1 expression levels based on a Combined Positive Score (CPS):

- CPS < 1
- CPS ≥ 1

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 32.

Photomicrographs

The included photomicrographs are of cervical cancer unless otherwise noted.

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

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

The data and biospecimens used in this project were provided by Contract Research Ltd (Charlestown, Nevis), National BioService LLC (Saint Petersburg, Russia), Sofia Bio LLC (New York, NY, USA), Assistance Publique-Hôpitaux de Paris (AP-HP; Paris, France) and contributions by AP-HP staff and Dr. Caroline Barau with appropriate ethics approval and through Azenta Life Sciences.

PD-L1 Overview

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

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

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

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

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

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

PD-L1 IHC 22C3 pharmDx Detects PD-L1 in Cervical Cancer Specimens

PD-L1 upregulation in cervical cancer is a biomarker for response to anti-PD-1 therapy. PD-L1 IHC 22C3 pharmDx was the only PD-L1 assay used in the KEYTRUDA® (pembrolizumab) clinical trial (KEYNOTE-826) to evaluate the relationship between PD-L1 expression and clinical efficacy. KEYTRUDA is a humanized monoclonal PD-1-blocking antibody.

The PD-1/PD-L1 Pathway

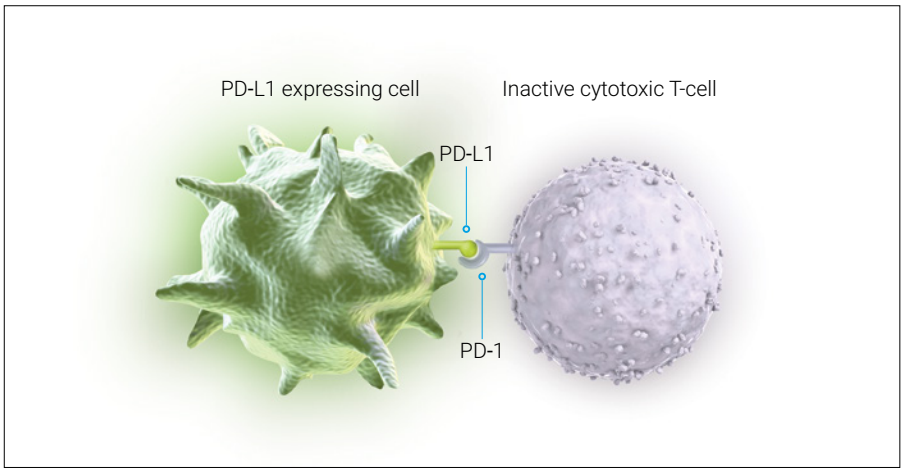


Figure 1: Inactivation of T-cells limits damage to normal tissue.

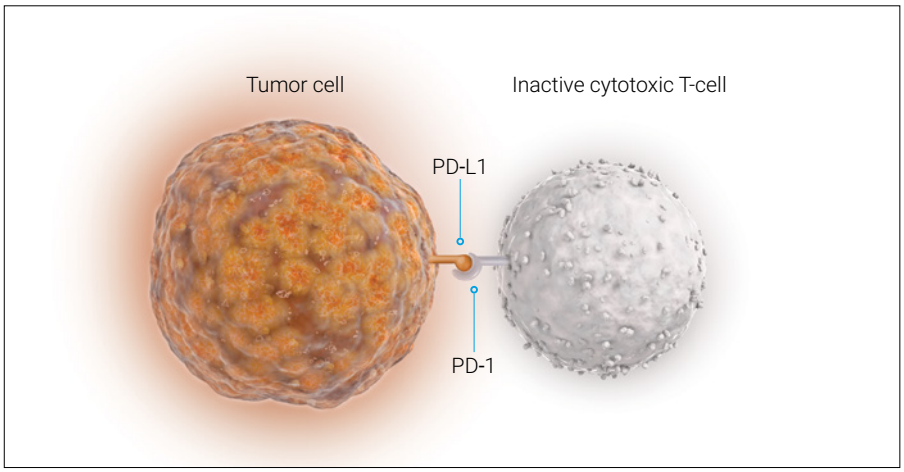


Figure 2: Inactivation of T-cells reduces tumor cell death and elimination.

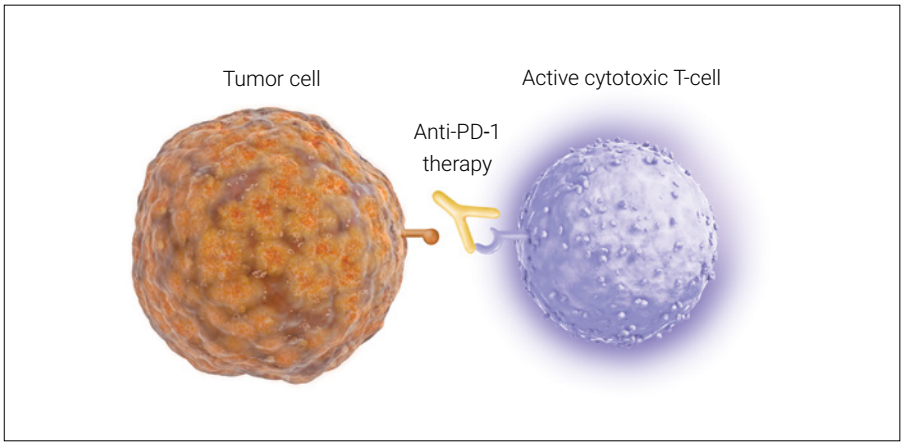


Figure 3: Blocking the PD-1/PD-L1 interaction helps to enable active T-cells and tumor cell death and elimination.

PD-L1 IHC 22C3 pharmDx Overview

What is PD-L1 IHC 22C3 pharmDx?

PD-L1 IHC 22C3 pharmDx is the only companion diagnostic indicated as an aid in identifying patients with cervical cancer 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 protein in FFPE cervical cancer 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.

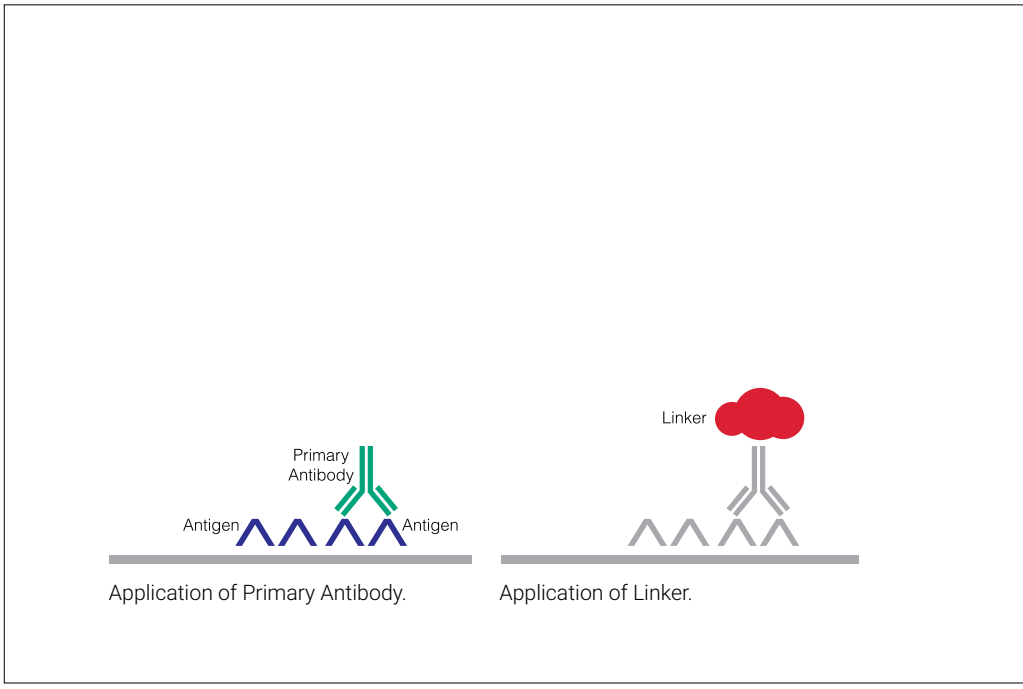


Figure 4: PD-L1 IHC 22C3 pharmDx staining procedure.

Kit Configuration (SK006)

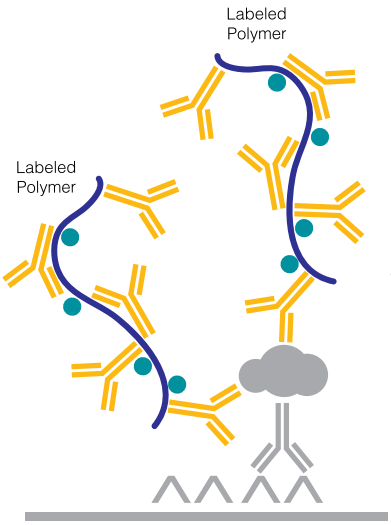
Figure 5: PD-L1 IHC 22C3 pharmDx components.

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

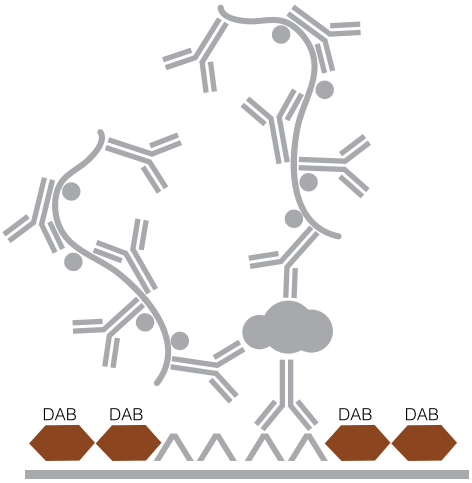
1. EnVision FLEX Target Retrieval Solution, Low pH (50×)
2. Peroxidase-blocking Reagent
3. Primary Antibody: Monoclonal Mouse Anti-PD-L1, Clone 22C3
4. Negative Control Reagent
5. Mouse LINKER
6. Visualization Reagent-HRP
7. DAB+ Substrate Buffer
8. DAB+ Chromogen
9. DAB Enhancer
10. PD-L1 IHC 22C3 pharmDx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

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

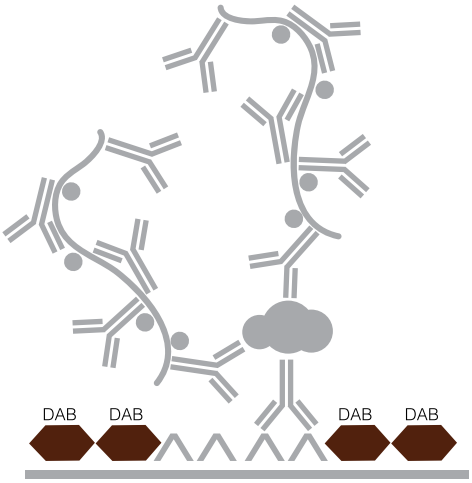
** Dr. AF Gazdar and Dr. JD Minna at NIH are acknowledged for their contribution in developing NCI-H226 (ATCC Number: CRL-5826™)*



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 with the Negative Control Reagent
- Serial section of each patient specimen stained with the NCR

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. For cervical cancer, NSCLC tissue may be used as a positive and/or negative control if no cervical cancer control tissue is available. 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 and tumor-associated mononuclear inflammatory cells (MICs: lymphocytes and macrophages). The ideal negative control tissue should demonstrate no staining on tumor cells and immune cells. However, because prevalence of PD-L1 expression on immune cells is high, a few staining immune cells are acceptable.

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. To preserve antigenicity, store tissue sections in the dark at 2–8 °C (preferred) and stain within 2 months of sectioning, or at room temperature up to 25 °C and stain within 1 month 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); the pH of 1× EnVision FLEX Target Retrieval Solution must be 6.1 ± 0.2. One 30 mL bottle of concentrate provides 1.5 L of 1× 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.

Additional EnVision FLEX Target Retrieval Solution, Low pH (50×), if required, is available as Code K8005.

EnVision FLEX Wash Buffer

Dilute EnVision FLEX Wash Buffer (20×) 1:20 (Code K8007) 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 (3-in-1) Procedure

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 medium. 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 tissues 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 2 months of sectioning when stored in the dark at 2–8 °C (preferred) or within 1 month when stored in the dark 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 30. 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 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

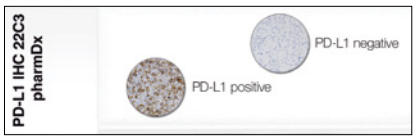


Figure 6: Each Control Cell Line Slide contains sections of cell pellets with positive and negative PD-L1 expression.

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 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

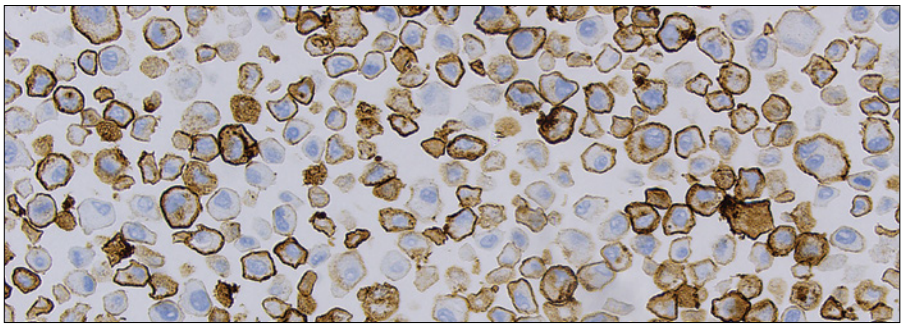


Figure 7: Positive cell pellet with acceptable staining of PD-L1 IHC 22C3 pharmDx Control Cell Line Slide (20× magnification).

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

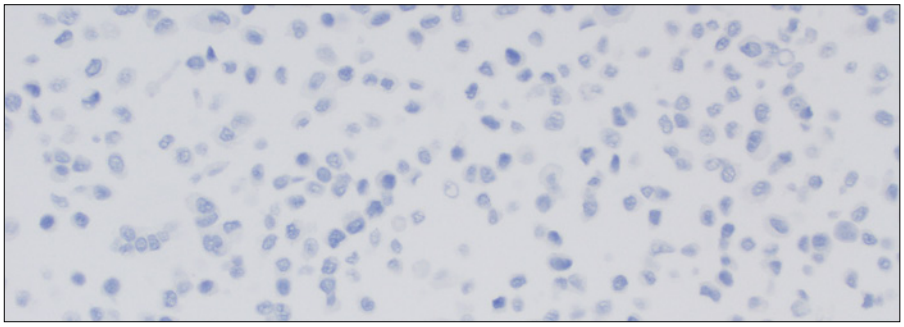


Figure 8: Negative cell pellet with no staining of PD-L1 IHC 22C3 pharmDx Control Cell Line Slide (20× magnification).

See the Control Cell Line (CCL) Appendix on page 72 for images of passing, borderline, and failing control cell line staining.

Positive and Negative In-house Control Tissues

Examine the cervical cancer positive control tissue slides to verify that the fixation method and epitope retrieval process are effective. NSCLC tissue may be used as a positive control if no cervical cancer control tissue is available. The positive control tissue slides should be stained with both PD-L1 primary antibody and Negative Control Reagent. The ideal positive control tissue provides a complete dynamic representation of weak-to-moderate staining of tumor cells and tumor-associated mononuclear inflammatory cells (MICs) (Figure 9). Known positive tissue controls should only be utilized for monitoring the correct performance of processed tissues and test reagents, not as an aid in formulating a specific diagnosis of patient samples. If staining of positive in-house control tissue is not satisfactory, all results with the patient specimen should be considered invalid.

- Requirements for slide stained with PD-L1: Presence of brown plasma membrane staining should be observed. Non-specific staining should be ≤ 1+
- Requirements for slide stained with Negative Control Reagent: No membrane staining. Non-specific staining should be ≤ 1+

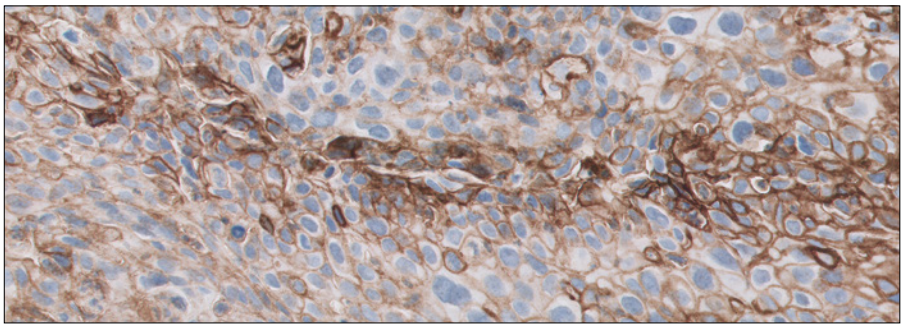


Figure 9: Positive in-house cervical cancer control tissue (20× magnification).

Examine the cervical cancer negative control tissue slides to verify the specificity of the labeling of the target antigen by the primary antibody. NSCLC tissue may be used as a negative control if no cervical cancer control tissue is available. The negative control tissue slides (known to be PD-L1 negative) should be stained with both PD-L1 primary antibody and Negative Control Reagent. The ideal negative control tissue should demonstrate no staining of tumor cells and immune cells (Figure 10). However, because prevalence of PD-L1 expression on immune cells is high, a few staining immune cells are acceptable.

Note: As an alternative, negative portions of the positive control tissue may serve as the negative control tissue, but this should be verified by the user.

If staining of negative in-house control tissue is not satisfactory, all results with the patient specimen should be considered invalid.

- Requirements for slide stained with PD-L1: No membrane staining in tumor cells. Non-specific staining should be ≤ 1+
- Requirements for slide stained with Negative Control Reagent: No membrane staining. Non-specific staining should be ≤ 1+

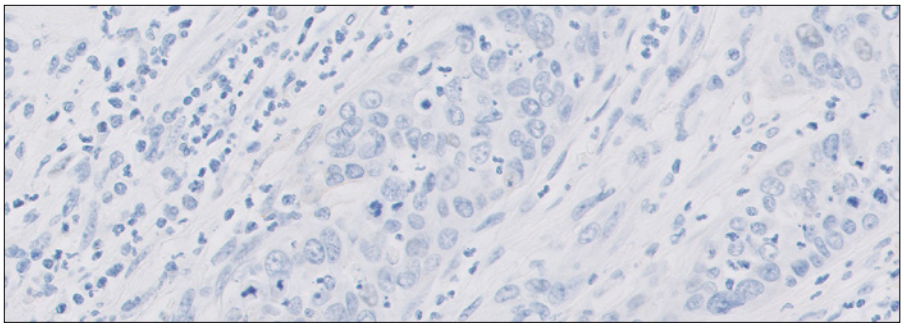


Figure 10: Negative in-house cervical cancer control tissue demonstrating lack of staining (20× magnification).

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.

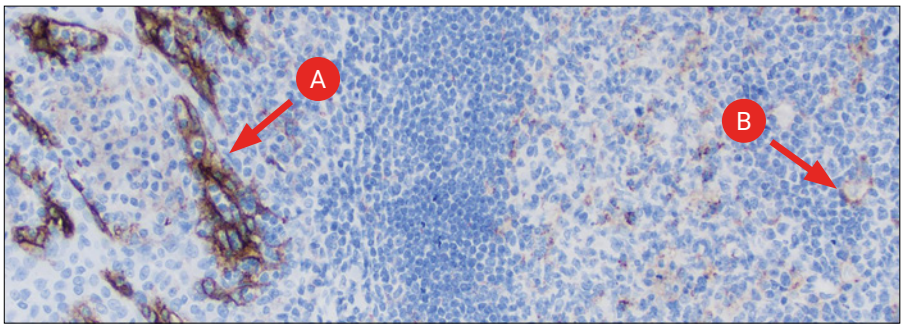


Figure 11: Tonsil stained with PD-L1 primary antibody exhibiting strong membrane staining in portions of the crypt epithelium (A) and weak-to-moderate membrane staining of follicular macrophages in the germinal centers (B) (10× magnification).

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 0 specific staining and ≤ 1+ non-specific 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.

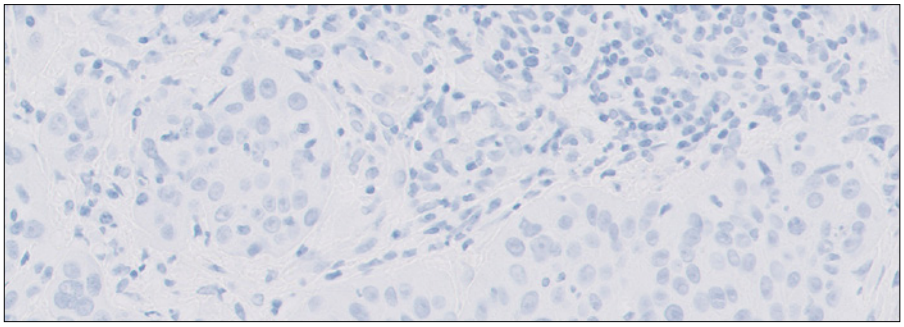


Figure 12: Cervical cancer tissue specimen stained with NCR (20× magnification).

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.

Combined Positive Score

Definition of Combined Positive Score (CPS)

PD-L1 expression in cervical cancer 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. Although the result of the calculation can exceed 100, the maximum score is defined as CPS 100.

CPS is defined accordingly:

$$\text{CPS} = \frac{\text{\# PD-L1 staining cells (tumor cells, lymphocytes, macrophages)}}{\text{Total \# of viable tumor cells}} \times 100$$

** Macrophages and histiocytes are considered the same cells*

CPS Numerator Inclusion and Exclusion Criteria

Any perceptible and convincing partial or complete linear membrane staining (≥ 1+) of viable tumor cells that is perceived as distinct from cytoplasmic staining is considered PD-L1 staining and should be included in the scoring.

Any membrane and/or cytoplasmic staining (≥ 1+) of lymphocytes and macrophages (mononuclear inflammatory cells, MICs) within tumor nests and/or adjacent supporting stroma is considered PD-L1 staining and should be included in the CPS numerator. Only MICs directly associated with the response against the tumor are scored.

See Tables 1 and 2 on page 26 for additional CPS inclusion/exclusion criteria.

Determining Combined Positive Score

- At lower magnifications, examine all well-preserved tumor areas. Evaluate overall areas of PD-L1 staining and non-staining tumor cells, keeping in mind that partial membrane staining or 1+ membrane staining may be difficult to see at low magnifications. Ensure there are at least 100 viable tumor cells in the sample
  - A minimum of 100 viable tumor cells must be present in the PD-L1 stained slide (biopsy / surgical specimens) for the specimen to be considered adequate for evaluation
- For 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 tumor cells for evaluation of PD-L1 expression
- At higher magnification (20×), evaluate PD-L1 expression and calculate CPS:
  - Determine the total number of viable tumor cells, both PD-L1 staining and non-staining (CPS denominator)
  - Determine the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) (CPS numerator; see Tables 1 and 2 on page 26 for additional CPS inclusion/exclusion criteria)
  - Calculate CPS
- Evaluation of membrane staining should be performed at no higher than 20× magnification. Slide reviewer should not perform the CPS calculation at 40× magnification

Table 1: CPS Numerator Inclusion/Exclusion Criteria for Cervical Cancer

Tissue Elements	Included in the Numerator	Excluded from the Numerator
Tumor Cells	Convincing partial or complete linear membrane staining (at any intensity) of viable invasive tumor cells	– Non-staining tumor cells – Tumor cells with only cytoplasmic staining – Dysplasia – Carcinoma in situ (CIS)
Immune Cells	Membrane and/or cytoplasmic* staining (at any intensity) of mononuclear inflammatory cells (MICs) within tumor nests and adjacent supporting stroma†, such as: – Lymphocytes (including lymphocyte aggregates) – Macrophages‡ Only MICs directly associated with the response to the tumor are scored	– Non-staining MICs – MICs associated with dysplasia and CIS – MICs associated with benign cells including squamous or glandular mucosa, cervical polyps, and microglandular hyperplasia – MICs (including lymphoid aggregates) associated with ulcers, and other processes not associated with the response to the tumor such as cervicitis – Neutrophils, eosinophils and plasma cells
Other Cells	Not included	– Benign cells including squamous or glandular mucosa, cervical polyps, and microglandular hyperplasia – Stromal cells (including fibroblasts) – Necrotic cells and/or cellular debris

** In MICs, membrane and cytoplasmic staining are often indistinguishable due to high nuclear to cytoplasmic ratio. Therefore, membrane and/or cytoplasmic staining of MICs is included in the score*

† Adjacent MICs are defined as being within the same 20× field as the tumor. However, MICs that are NOT directly associated with the response to the tumor should be excluded

‡ Macrophages and histiocytes are considered the same cells

Table 2: CPS Denominator Inclusion/Exclusion Criteria for Cervical Cancer

Tissue Elements	Included in the Denominator	Excluded from the Denominator
Tumor Cells	All viable tumor cells	– Any necrotic or non-viable tumor cells – Carcinoma in situ (CIS) – Dysplasia
Immune Cells	Not included	All immune cells of any type
Other Cells	Not included	– Benign cells including squamous or glandular mucosa, cervical polyps, and microglandular hyperplasia – Stromal cells (including fibroblasts) – Necrotic cells and/or cellular debris

Suggested Methods

Agilent recommends that scoring be performed within the context of the pathologist's past experience and best judgment in interpreting IHC stains. We offer three different examples of techniques that may be used when determining the respective Combined Positive Scores (CPS) of various staining patterns.

The entire IHC slide should be reviewed to determine which of the following example techniques may be used.

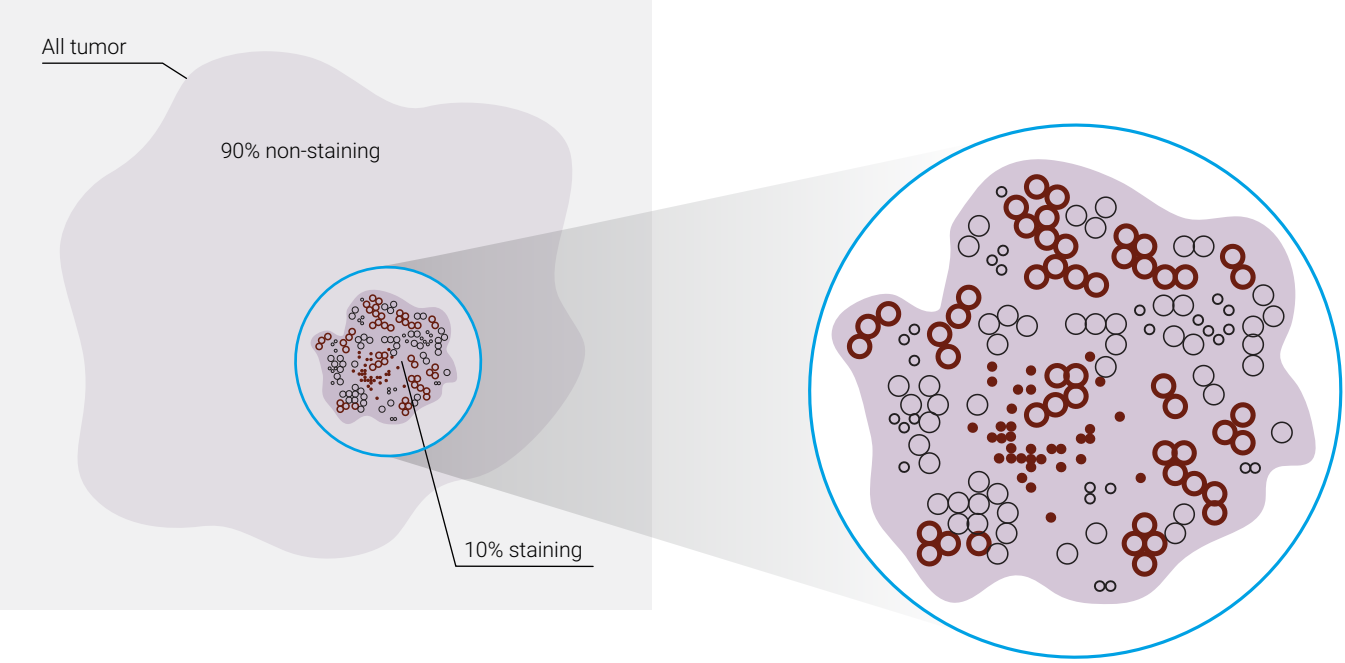
Example 1: Calculation of Combined Positive Score Based on a Small PD-L1 Staining Area

First: Evaluate the tumor area for perceptible and convincing staining as described in "Determining Combined Positive Score" on page 25.

Assessment: *10% of area shows staining, 90% of area shows no staining*

Second: Evaluate the area of staining to estimate the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages).

Assessment: *There are approximately 100 viable tumor cells and about 80 PD-L1 staining cells (per the CPS numerator)*



Calculate the Combined Positive Score of the entire tumor area:

Assessment:

CPS of area with staining:

$$\text{CPS} = \frac{\text{\# PD-L1 staining cells}^{\S}}{\text{Total \# of viable tumor cells}} \times 100 = \frac{\sim 80 \text{ PD-L1 staining cells}}{100 \text{ tumor cells}} \times 100 = 80$$

CPS of entire tumor area: 10% × 80 ≈ CPS 8

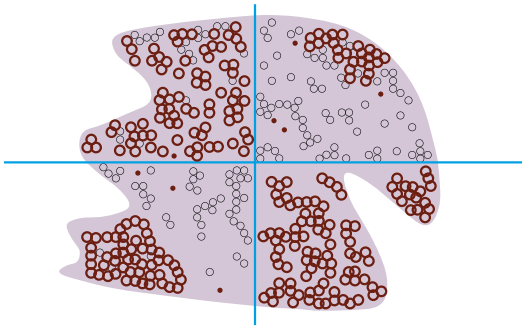
Clinical Interpretation: CPS ≥ 1

§ Including tumor cells, lymphocytes, macrophages

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

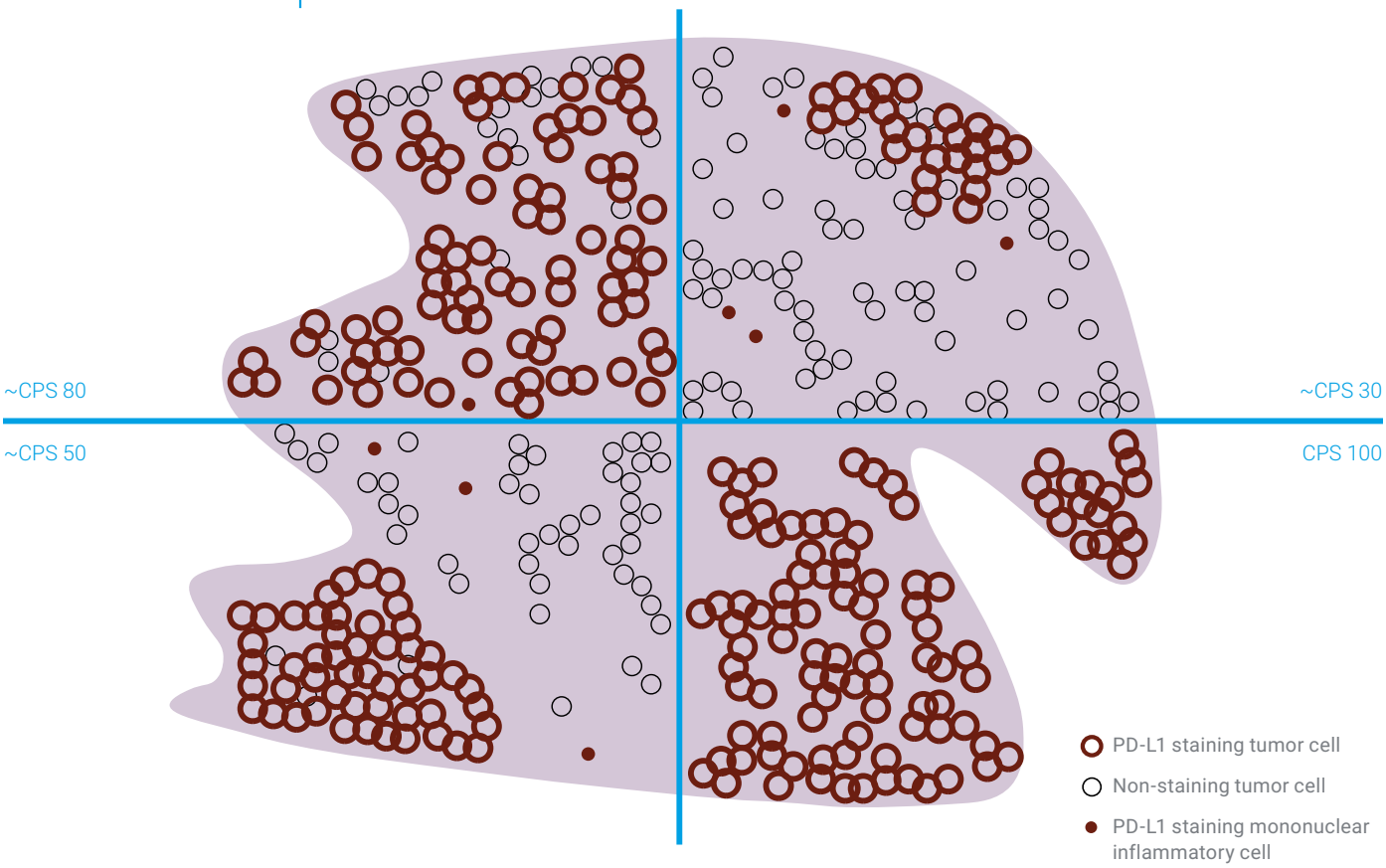
Example 2: Calculation of Combined Positive Score Based on a Heterogeneous PD-L1 Staining Area

First: Visually divide the tumor area into regions with equal numbers of tumor cells.



Second: Observe each region and estimate the total number of viable tumor cells and PD-L1 staining cells (tumor cells, lymphocytes, macrophages). Calculate the Combined Positive Score for each region.

Assessment: *The four sections have ~80, ~30, ~50, and 100 PD-L1 staining cells (tumor cells, lymphocytes, macrophages). Each section has a total of 100 tumor cells (including PD-L1 staining cells). The CPS for each section: ~CPS 80, ~CPS 30, ~CPS 50, and CPS 100*



Calculate the Combined Positive Score of the entire tumor area:

Assessment:

Combined Positive Score:

(80 + 30 + 50 + 100) / 4 ≈ CPS 65

$$\text{CPS} = \frac{\text{\# PD-L1 staining cells (tumor cells, lymphocytes, macrophages)}}{\text{Total \# of viable tumor cells}} \times 100$$

Clinical Interpretation: CPS ≥ 1

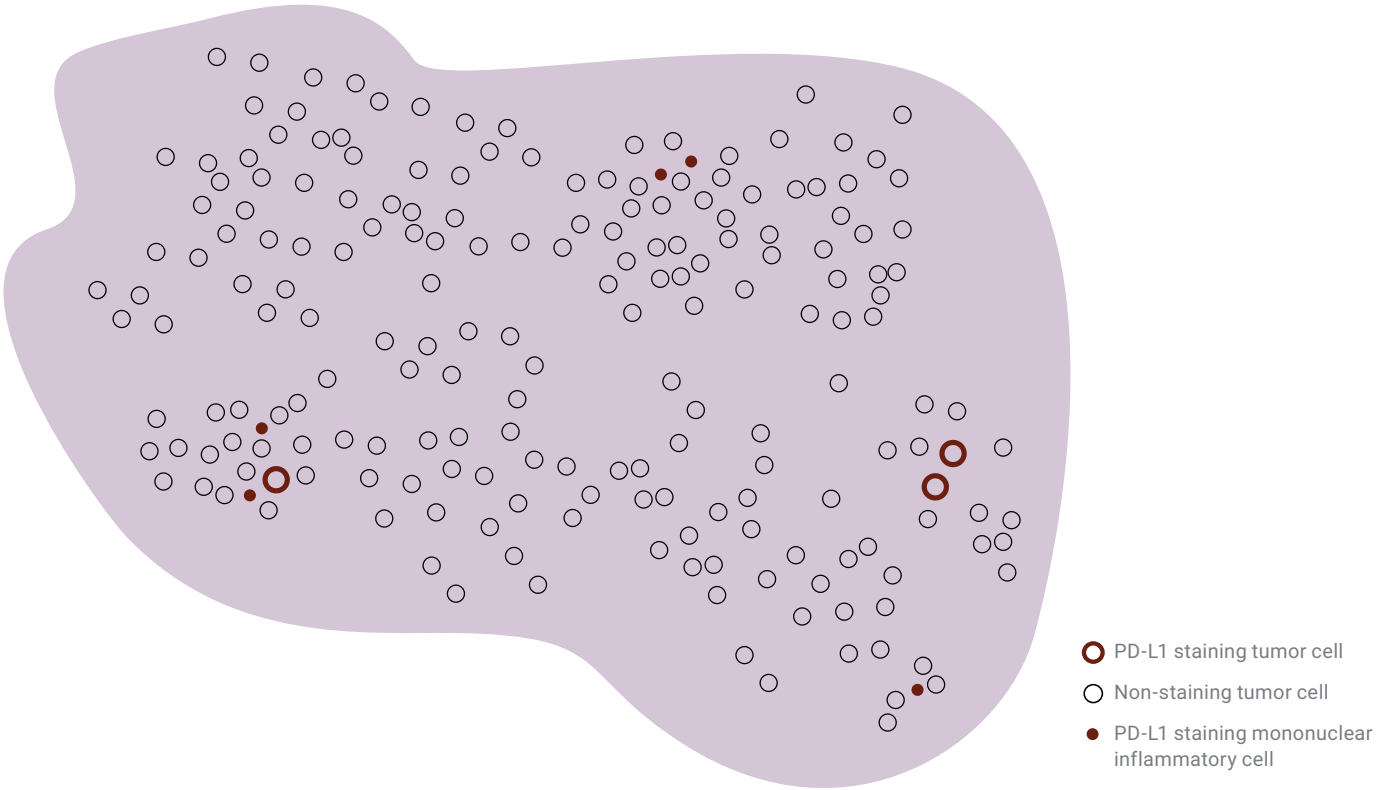
Figure 15: Example with heterogeneous PD-L1 staining area.

Example 3: Calculation of Combined Positive Score for a Near-Cutoff Specimen

First: Evaluate the specimen for perceptible and convincing staining as described in "Determining Combined Positive Score" on page 25.

Second: Confirm that there is no staining in areas that appeared void of staining at lower magnifications. Evaluate all staining areas and estimate the total number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages). Then re-evaluate the entire specimen (staining and non-staining areas) and estimate the total number of viable tumor cells (PD-L1 staining and non-staining tumor cells). Calculate the Combined Positive Score.

Assessment: *Four areas of the tumor specimen have convincing staining. There are 8 PD-L1 staining cells (tumor cells, lymphocytes, macrophages) in the four staining areas. There are approximately 200 viable tumor cells present in the entire specimen*



Calculate the Combined Positive Score of the entire tumor area:

Assessment:

Combined Positive Score:

$$\text{CPS} = \frac{\text{\# PD-L1 staining cells*}}{\text{Total \# of viable tumor cells}} \times 100 = \frac{\text{8 PD-L1 staining cells}}{\text{200 tumor cells}} \times 100 = 4$$

Clinical Interpretation: CPS ≥ 1

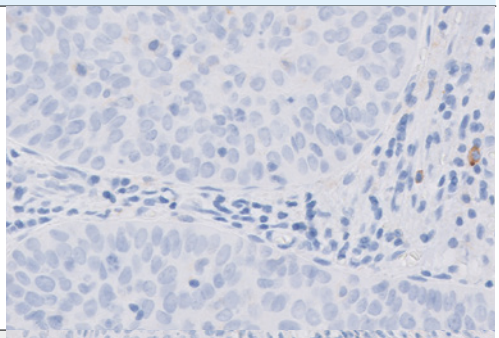
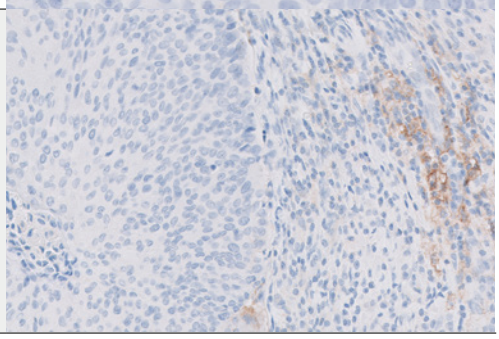
** Including tumor cells, lymphocytes, macrophages*

Figure 16: Example of near-cutoff specimen (CPS range of greater than 0 but less than or equal to 10).

Interpretation of CPS

The Combined Positive Score (CPS) determines the PD-L1 expression level of the specimen. See the table below for scoring interpretation examples.

Table 3: CPS and Corresponding PD-L1 Expression Levels

CPS	PD-L1 Expression Level	Image (20× magnification)
< 1	CPS is less than 1	
≥ 1	CPS is greater than or equal to 1	

Identifying Patients with Cervical Cancer for Treatment

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

Clinical Validation of PD-L1 IHC 22C3 pharmDx in Previously Untreated Patients with Cervical Cancer

The clinical validity of PD-L1 IHC 22C3 pharmDx in evaluating PD-L1 expression in previously untreated patients with cervical cancer is based on the KEYTRUDA KEYNOTE-826 study sponsored by Merck Sharp & Dohme LLC. Specimens from patients with persistent, recurrent, or first-line metastatic cervical cancer who had not been treated with chemotherapy except when used concurrently as a radio-sensitizing agent were tested for PD-L1 expression using PD-L1 IHC 22C3 pharmDx. Eighty-nine percent of enrolled patients had tumors that expressed PD-L1 with a Combined Positive Score (CPS) of greater than or equal to 1 (CPS ≥ 1) (Table 4). Clinical efficacy of KEYTRUDA treatment is presented in the Clinical Performance Evaluation section on pages 85–89.

Table 4: PD-L1 Prevalence in Patients with Persistent, Recurrent, or First-line Metastatic Cervical Cancer Enrolled in KEYNOTE-826

PD-L1 Expression	CPS < 1	CPS ≥ 1
Prevalence % (n)	11% (69)	89% (548)

PD-L1 IHC 22C3 pharmDx Testing Scheme

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

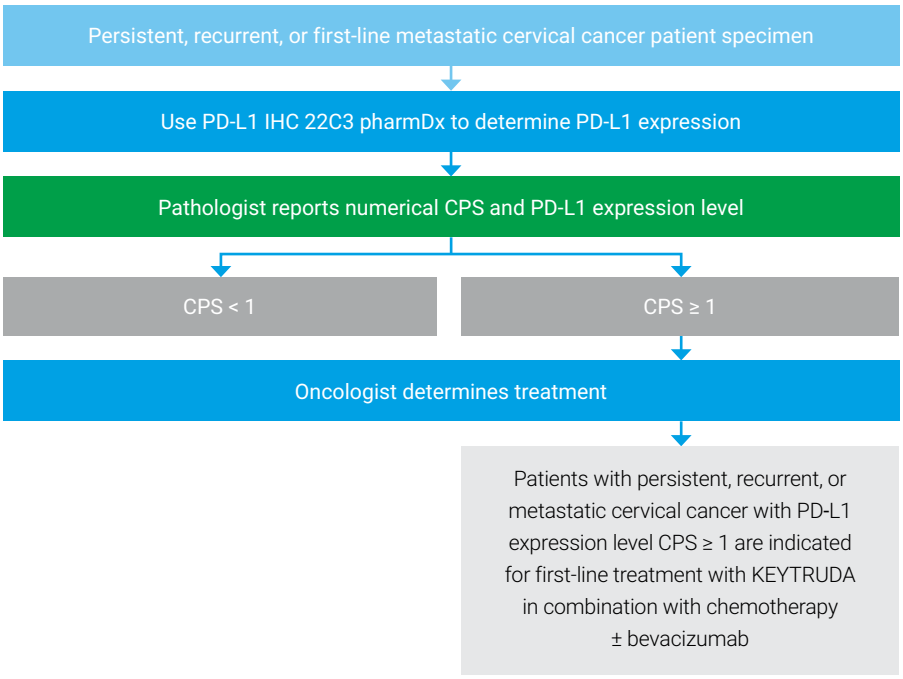


Figure 17: Testing scheme for PD-L1 IHC 22C3 pharmDx.

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 Testing Results

Control Cell Line Slide Results: Pass: ☐ Fail: ☐

Adequate Tumor Cells Present (≥ 100 cells): Yes: ☐ No: ☐

PD-L1 IHC 22C3 pharmDx Result to Treating Physician

Combined Positive Score: _____

CPS < 1: ☐ CPS ≥ 1: ☐

Comments to Treating Physician:

– KEYTRUDA® (pembrolizumab), in combination with chemotherapy ± bevacizumab, is indicated for the first-line treatment of patients with persistent, recurrent or metastatic cervical cancer and whose tumors express PD-L1 with a CPS ≥ 1. See the KEYTRUDA prescribing information for details.

Combined Positive Score Summary and Examples

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

By definition, PD-L1 staining cells in cervical cancer are:

- Viable tumor cells with perceptible and convincing partial or complete linear membrane staining (at any intensity) that is perceived distinct from cytoplasmic staining
- Lymphocytes and macrophages (mononuclear inflammatory cells, MICs) within the tumor nests and/or adjacent supporting stroma with membrane and/or cytoplasmic staining (at any intensity). MICs must be directly associated with the response against the tumor

PD-L1 expression status in cervical cancer is determined by 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.

$$\text{CPS} = \frac{\text{\# PD-L1 staining cells (tumor cells, lymphocytes, macrophages)}}{\text{Total \# of viable tumor cells}} \times 100$$

This section will define and illustrate scoring inclusions and exclusions for accurate determination of Combined Positive Score. All images are cervical cancer unless otherwise noted in the figure caption.

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

Cells Included in the Combined Positive Score (CPS)

Tumor cells, lymphocytes, and macrophages exhibiting appropriate PD-L1 expression are defined as PD-L1 staining cells. All PD-L1 staining cells are included in the CPS numerator for determination of the Combined Positive Score (see Tables 1 and 2 on page 26 for additional CPS inclusion/exclusion criteria). Below is an image guide to aid in accurately determining CPS in cervical cancer specimens. All images are cervical cancer unless otherwise noted in the figure caption.

Tumor Cells

Tumor Cell Size

Cervical cancer includes different morphologies and tumor cell sizes that can impact the Combined Positive Score (CPS) by increasing or decreasing the total number of tumor cells that are included in the denominator. Well-differentiated squamous cell carcinoma may exhibit larger tumor cells with abundant keratinous cytoplasm and will commonly have fewer cells per 20× field. Alternatively, a poorly-differentiated, basaloid pattern will commonly have a higher number of tumor cells per 20× field due to the smaller size and scant cytoplasm of the tumor cells. The more tumor cells included in the denominator, the greater the number of PD-L1 staining tumor cells, lymphocytes, and macrophages that are needed in the numerator to bring the overall score to CPS 1 or above. As a guideline, if tumor cells are 20 µm in diameter and completely fill a 20× field, there would be approximately 2500 tumor cells in that field.

Small Cell Size

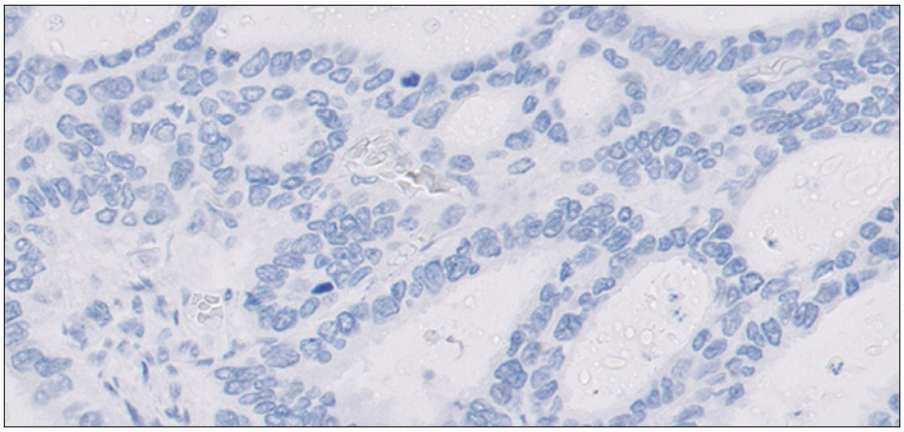


Figure 18a: Cervical adenocarcinoma specimen with small sized tumor cells (20× magnification).

Medium Cell Size

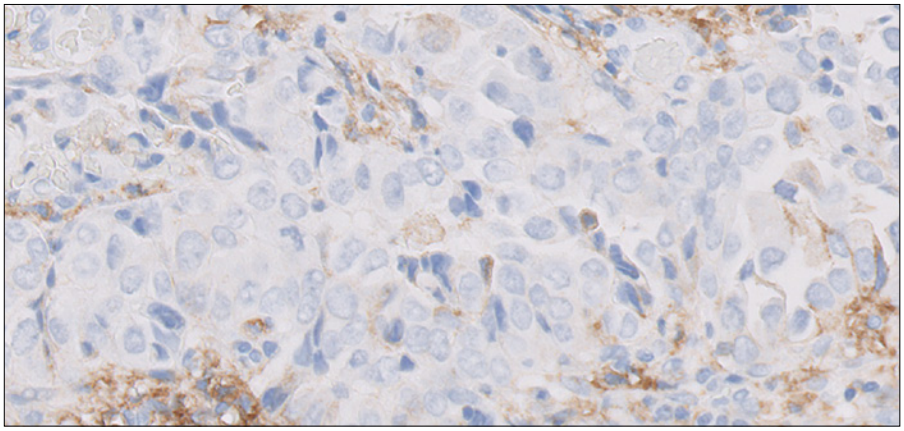


Figure 18b: Cervical adenocarcinoma specimen with medium sized tumor cells (20× magnification).

Large Cell Size

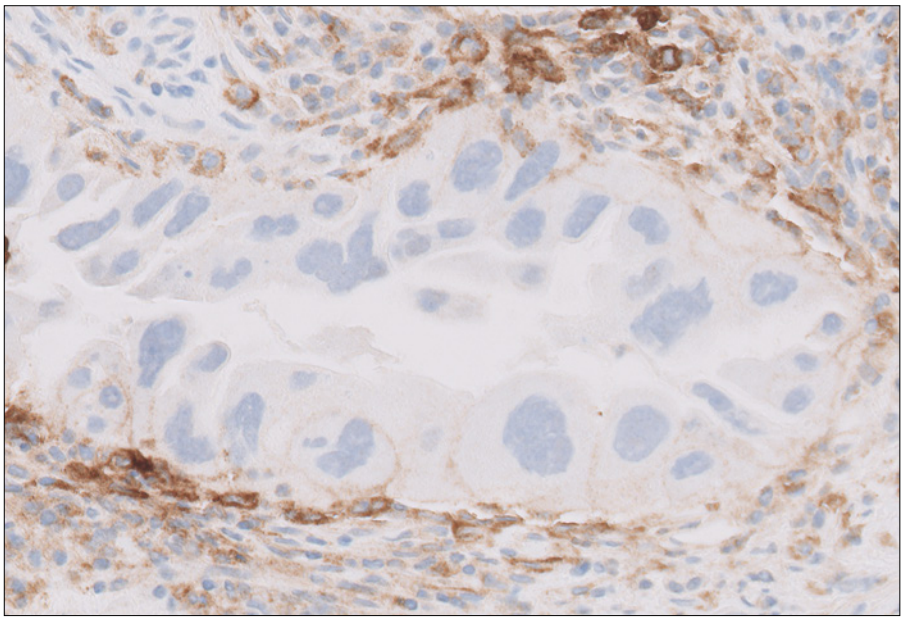


Figure 18c: Cervical adenocarcinoma specimen with large sized tumor cells (20× magnification).

Key Point

The size of tumor cells can impact the CPS by increasing or decreasing the total number of tumor cells in the denominator

Tumor and Immune Cells That Are Difficult to Distinguish

Tumor cells and tumor-associated lymphocytes and macrophages may be difficult to distinguish from each other when examining the slide with PD-L1 antibody staining due to small tumor cell size and staining characteristics. It is recommended to use the corresponding H&E slide to distinguish cell types. This is especially important when determining the denominator.

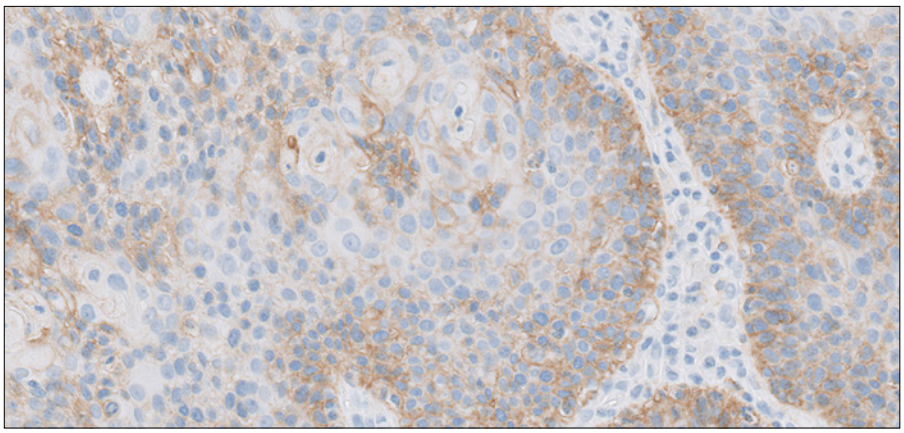


Figure 19a: Tumor and tumor-associated mononuclear inflammatory cells (MICs) are difficult to distinguish from each other and exhibit PD-L1 primary antibody staining (20× magnification).

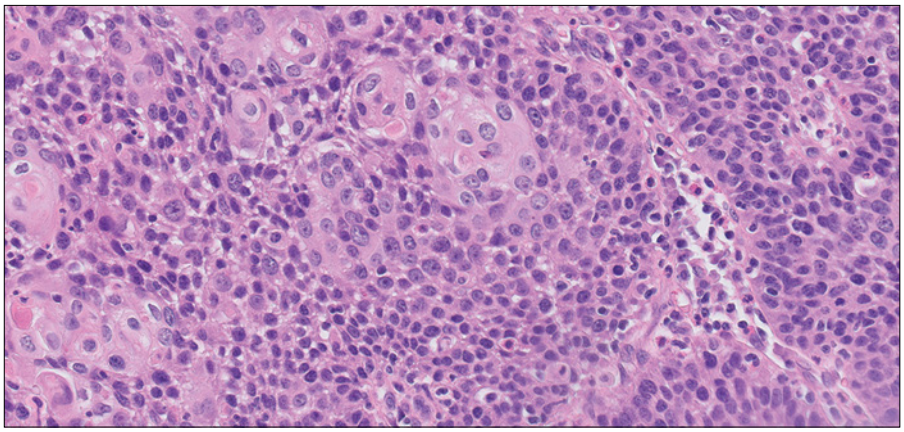


Figure 19b: Corresponding H&E to reference when tumor and tumor-associated mononuclear inflammatory cells (MICs) are difficult to distinguish from each other (20× magnification).

Key Point

Utilize the H&E slide when it is difficult to distinguish tumor cells from immune cells

Linear Membrane Staining

Tumor cells exhibiting convincing partial and/or complete smooth or granular linear membrane staining are considered PD-L1 staining cells. Convincing linear membrane staining can be present at any intensity and must be convincing at no higher than 20× magnification.

Convincing staining of tumor cells (linear membrane staining) is often heterogeneous, with various staining intensities present.

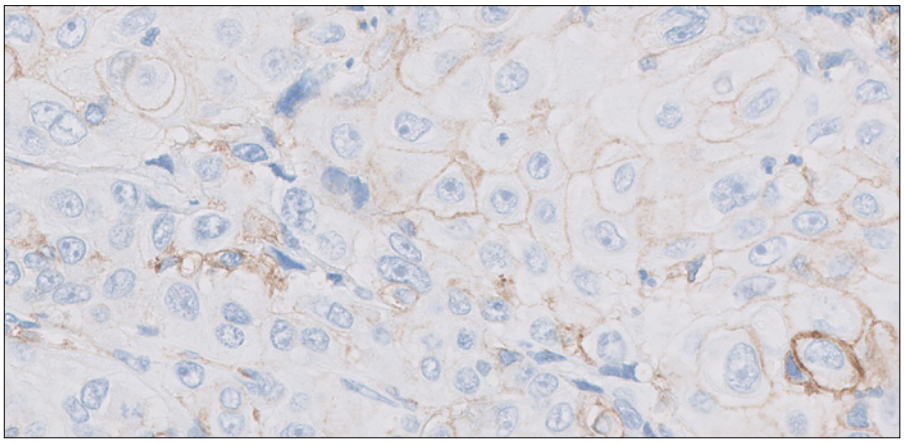


Figure 20a: Cervical squamous cell carcinoma specimen stained with PD-L1 primary antibody exhibiting 1+ linear membrane staining of tumor cells (20× magnification).

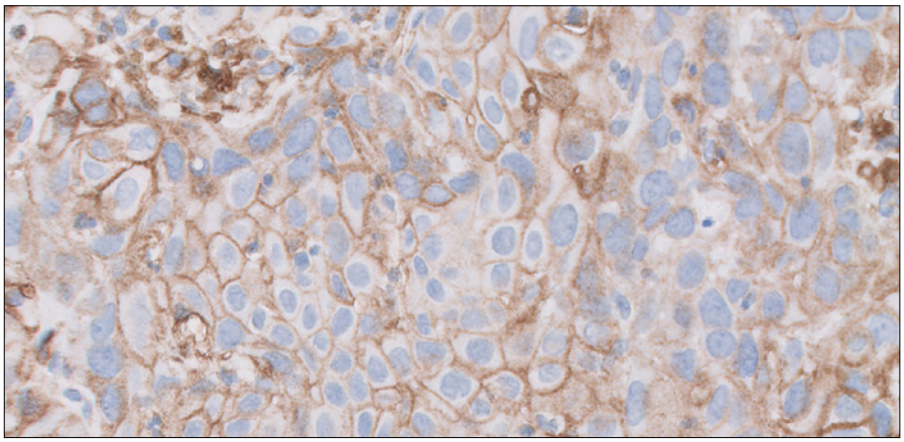


Figure 20b: Cervical squamous cell carcinoma specimen stained with PD-L1 primary antibody exhibiting 2+ linear membrane staining of tumor cells (20× magnification).

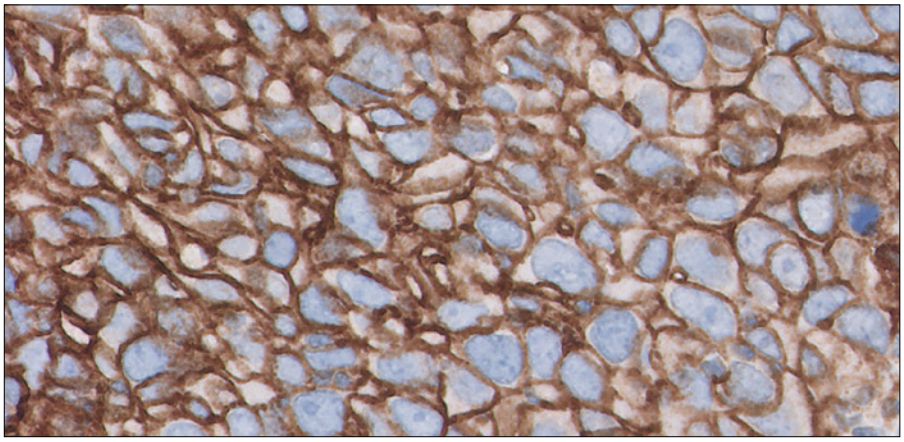


Figure 20c: Cervical squamous cell carcinoma specimen stained with PD-L1 primary antibody exhibiting 3+ linear membrane staining of tumor cells (20× magnification).

Key point

Convincing linear membrane staining of tumor cells at any intensity should be included in the score

Partial and Complete Linear Membrane Staining

Tumor cells can exhibit partial or complete linear membrane staining. Any tumor cells with partial or complete linear membrane staining that is perceptible and convincing at 20× magnification should be included in the CPS numerator.

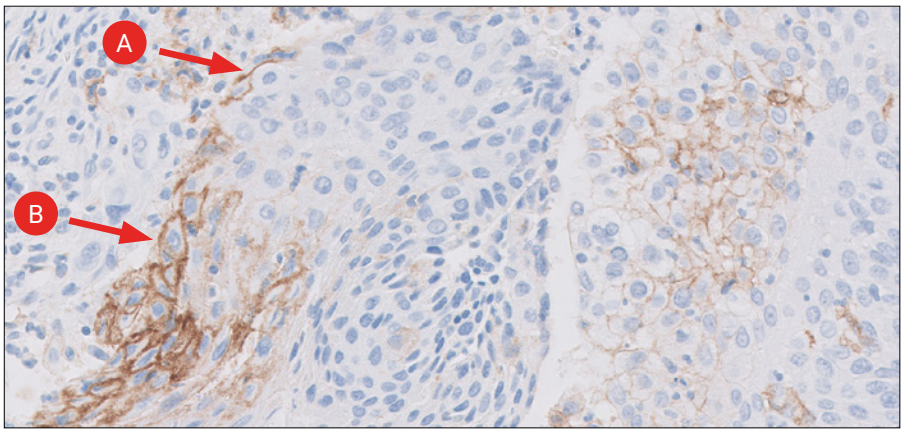


Figure 21: Cervical squamous cell carcinoma specimen stained with PD-L1 primary antibody exhibiting partial (A) and complete (B) linear membrane staining of tumor cells (20× magnification).

Key point

Convincing partial or complete linear membrane staining of tumor cells should be included in the score

Linear Membrane and Cytoplasmic Staining

Tumor cells with both convincing linear membrane staining (≥ 1+ intensity) and cytoplasmic staining at 20× magnification should be included in the CPS numerator. Tumor cells exhibiting only cytoplasmic staining are excluded from the CPS numerator, as this is considered non-specific staining. If linear membrane staining is distinct from cytoplasmic staining, then the cell should be included in the score.

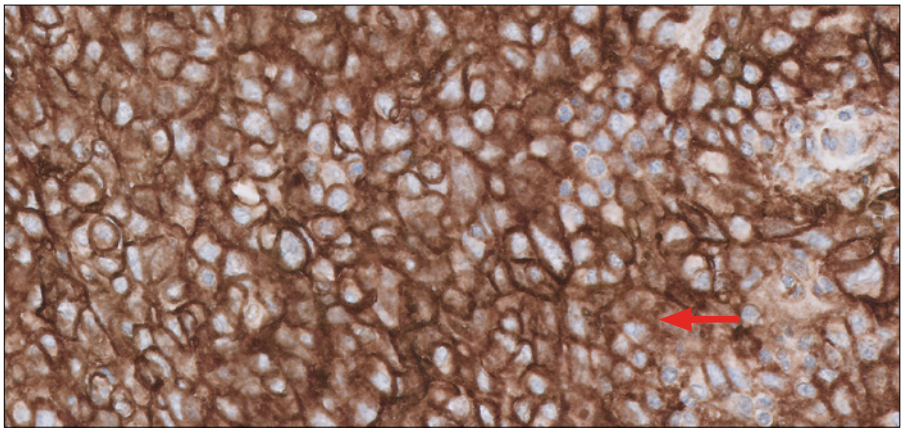


Figure 22: Cervical squamous cell carcinoma specimen stained with PD-L1 primary antibody exhibiting linear membrane staining distinct from cytoplasmic staining (arrow) (20× magnification).

Key point

Tumor cells exhibiting convincing linear membrane staining that is distinct from cytoplasmic staining are included in the score

Granular Staining

Linear membrane staining of tumor cells can be smooth or granular. Tumor cells can exhibit a granular membrane staining pattern where membrane and cytoplasmic staining are difficult to distinguish. Only perceptible and convincing linear membrane staining of tumor cells (≥ 1+ intensity) observed at no higher than 20× magnification should be included in the CPS numerator.

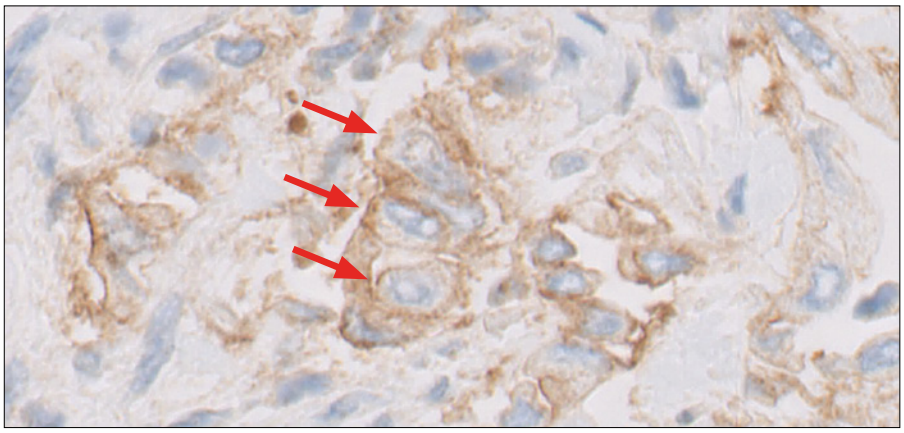


Figure 23: Cervical adenocarcinoma specimen stained with PD-L1 primary antibody exhibiting granular membrane staining of tumor cells (arrows) (20× magnification).

Key Point

Granular staining of tumor cells must exhibit a perceptible and convincing linear membrane pattern to be included in the CPS numerator

Multinucleate Tumor Cells

Some tumor cells in cervical cancer may be multinucleate and each multinucleate tumor cell should be counted as one cell. The same rules should apply for inclusion in the numerator and denominator: all viable tumor cells should be included in the denominator and all tumor cells with partial or complete linear membrane staining should be included in the numerator.

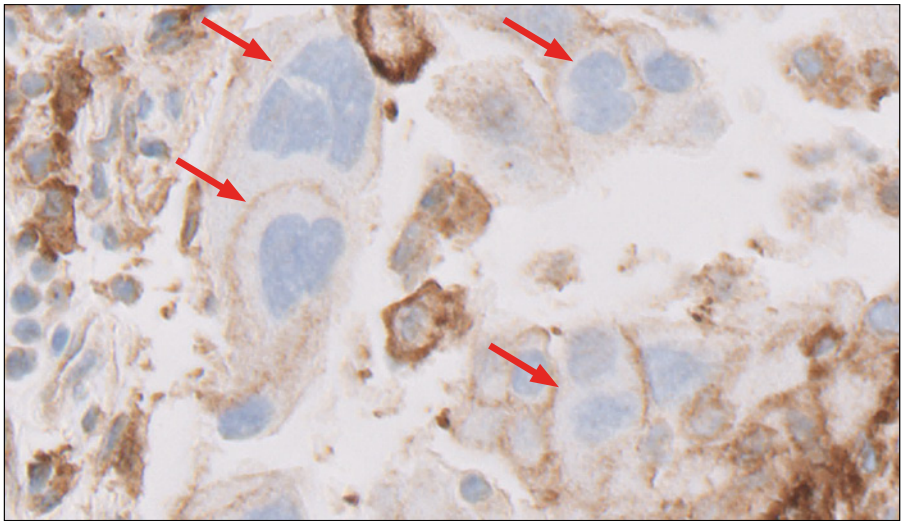


Figure 24: Cervical adenocarcinoma specimen stained with PD-L1 primary antibody exhibiting staining of multinucleate tumor cells (arrows) (20× magnification).

Key Point

Multinucleate tumor cells can be seen in cervical cancer and follow the same criteria for inclusion/exclusion as mononucleate tumor cells

Keratinizing Tumor Cells

Nucleated keratinizing tumor cells are considered viable and should be included in the CPS denominator. Viable keratinizing tumor cells exhibiting convincing linear membrane staining should be included in the CPS numerator.

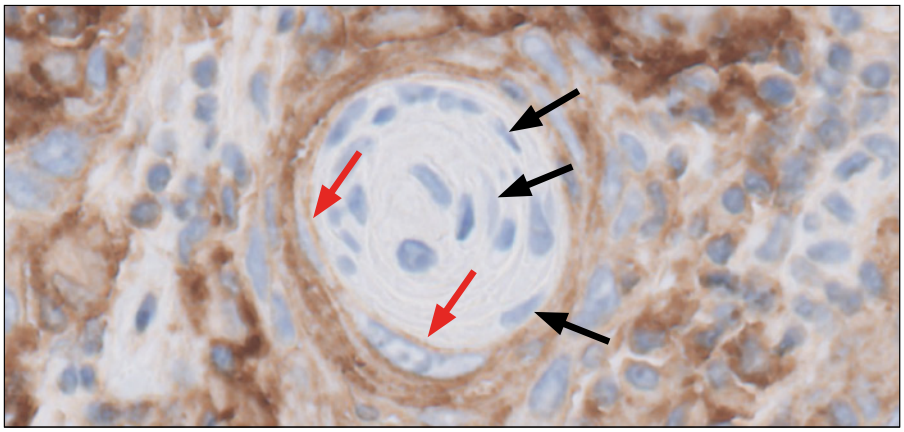


Figure 25: Viable PD-L1 staining and non-staining keratinizing tumor: nucleated keratinizing tumor cells are considered viable (black arrows) and should be included in the CPS denominator, and if staining (red arrows), should also be included in the CPS numerator (20× magnification).

Intravascular Tumor Cells

Viable intravascular tumor cells should be included in the CPS denominator, and if exhibiting convincing linear membrane staining, should be included in the CPS numerator.

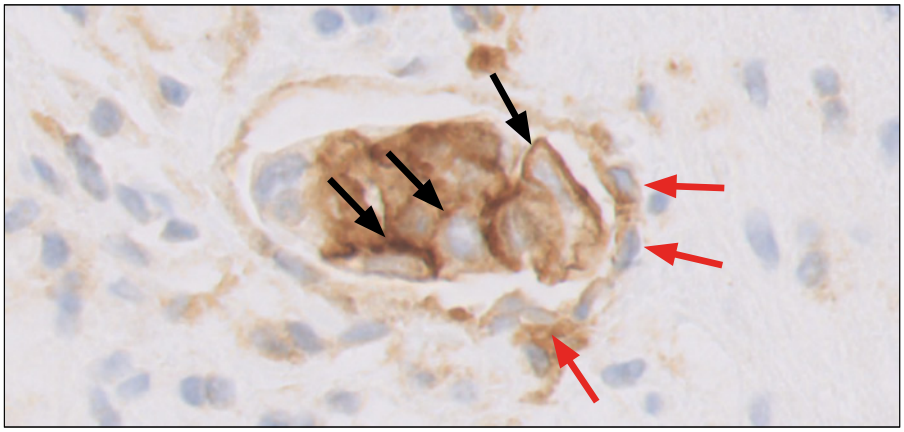


Figure 26: PD-L1 staining intravascular tumor cells (black arrows) should be included in the CPS numerator and denominator. Note: PD-L1 staining benign endothelial cells (red arrows) should be excluded from the CPS calculation (20× magnification).

Immune Cells

Tumor-associated Mononuclear Inflammatory Cells (MICs)

Tumor-associated lymphocytes and macrophages (mononuclear inflammatory cells, MICs) exhibiting membrane and/or cytoplasmic staining at a 20× magnification (≥ 1+ intensity) are considered PD-L1 staining cells and should be included in the CPS numerator. Tumor-associated MICs are present within the tumor nests and/or adjacent supporting stroma and are directly associated with the response against the tumor.

Staining of tumor-associated lymphocytes and macrophages (membrane and/or cytoplasmic) is often heterogeneous, with various staining intensities present.

Note: PD-L1 staining lymphocytes often have indistinguishable membrane and cytoplasmic staining due to a high nuclear to cytoplasmic ratio; PD-L1 staining macrophages often have distinct membrane staining and low cytoplasmic staining. All PD-L1 staining tumor-associated MICs should be included in the CPS numerator.

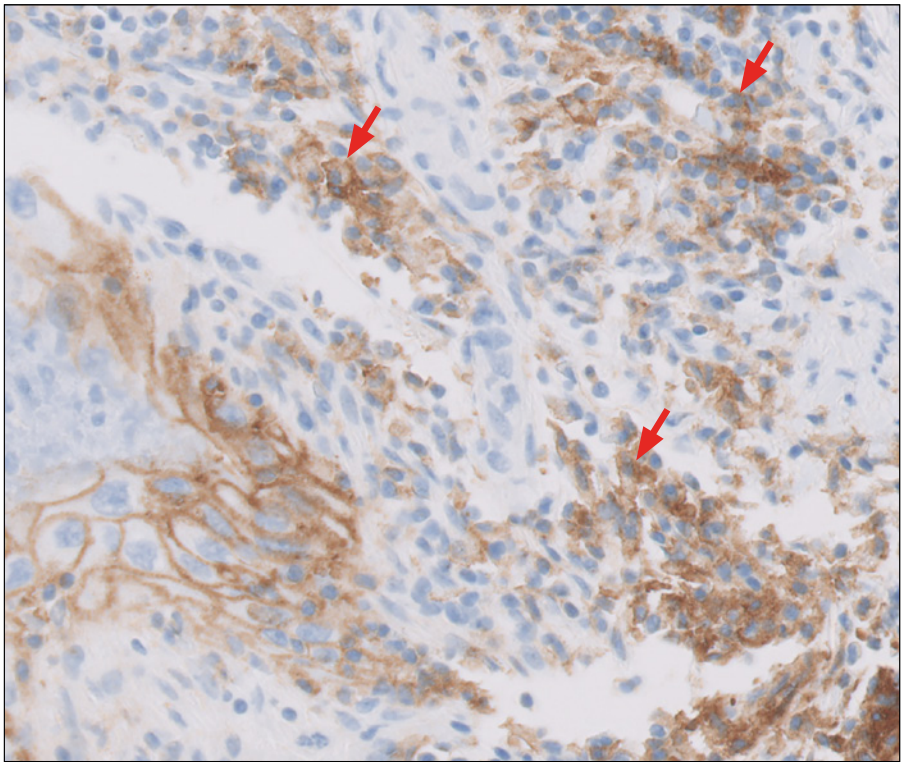


Figure 27a: Cervical adenocarcinoma specimen stained with PD-L1 primary antibody exhibiting staining of tumor-associated lymphocytes (arrows) (20× magnification).

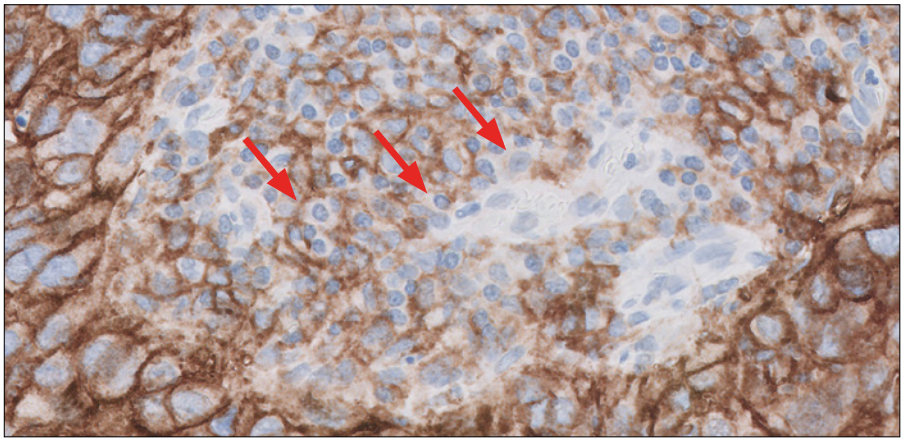


Figure 27b: Cervical squamous cell carcinoma specimen stained with PD-L1 primary antibody exhibiting staining of tumor-associated macrophages (arrows) (20× magnification).

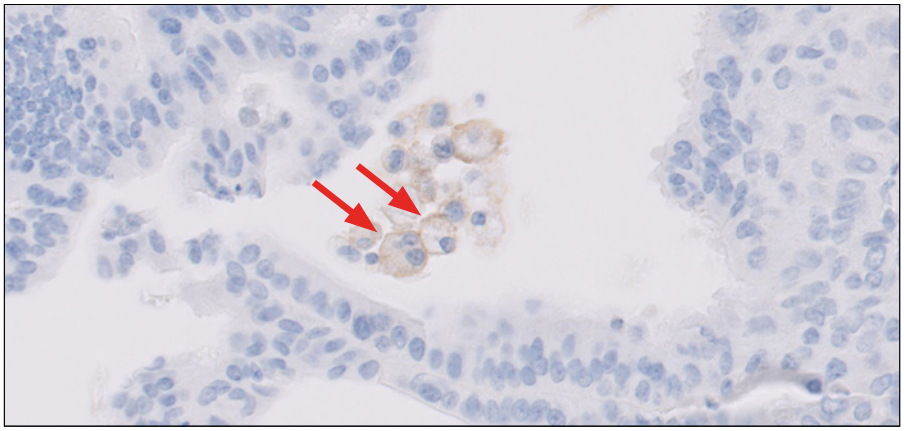


Figure 27c: Cervical adenocarcinoma specimen stained with PD-L1 primary antibody exhibiting PD-L1 staining intraluminal macrophages within tumor that should be included in the CPS numerator (arrows) (20× magnification).

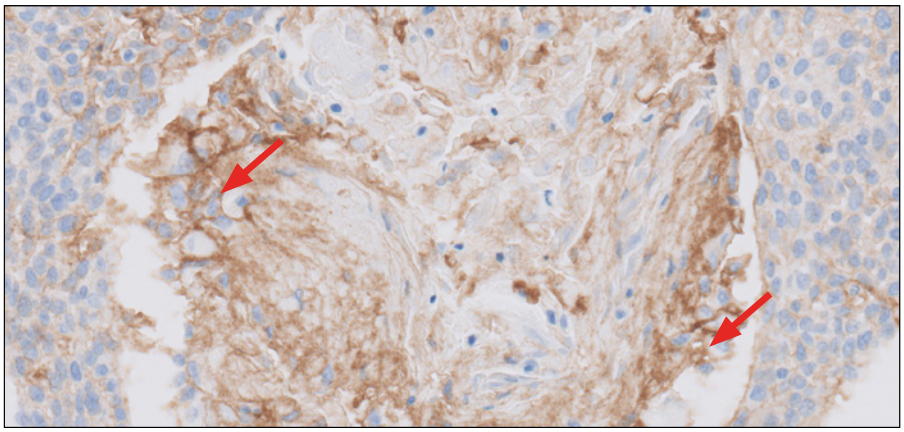


Figure 27d: Cervical squamous cell carcinoma specimen stained with PD-L1 primary antibody exhibiting staining of tumor-associated mononuclear inflammatory cells (MICs) within necrosis but adjacent to viable tumor cells and should be included in the CPS numerator (arrows) (20× magnification).

Multinucleated Giant Cells

Multinucleate giant cells can be seen in cervical cancer and, if PD-L1 staining is present on these cells, each multinucleate giant cell should be counted as one cell and included in the numerator.

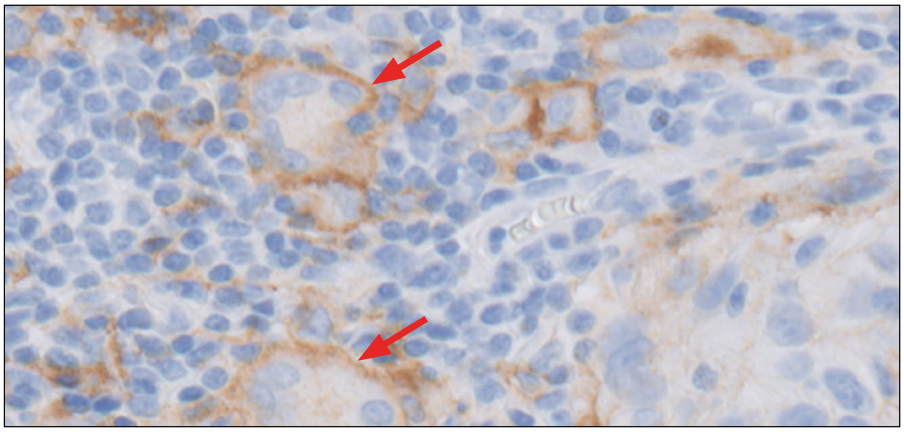


Figure 28: Cervical adenocarcinoma specimen stained with PD-L1 primary antibody exhibiting staining of tumor-associated multinucleated giant cells (arrows) (20× magnification).

Key point

Tumor-associated lymphocytes and macrophages with membrane and/or cytoplasmic staining should be included in the CPS numerator

Immune Cell Inclusion/Exclusion: 20× Rule

PD-L1 staining mononuclear inflammatory cells (MICs) must be directly associated with the response against the tumor to be included in the CPS numerator. MICs are considered tumor-associated if they are present within the tumor nests and/or adjacent supporting stroma within a 20× magnification field of view. In cases where it is difficult to tell if MICs are tumor-associated, the following is suggested as a guideline:

Move the slide so that the tumor is in the approximate center of a 20× field. Immune cells surrounding the tumor in this field should be included in scoring. Immune cells outside of this field should be excluded from scoring as long as they do not surround neighboring tumor cells. See Figures 29a–29c for an example of determining which MICs are included in the CPS numerator.

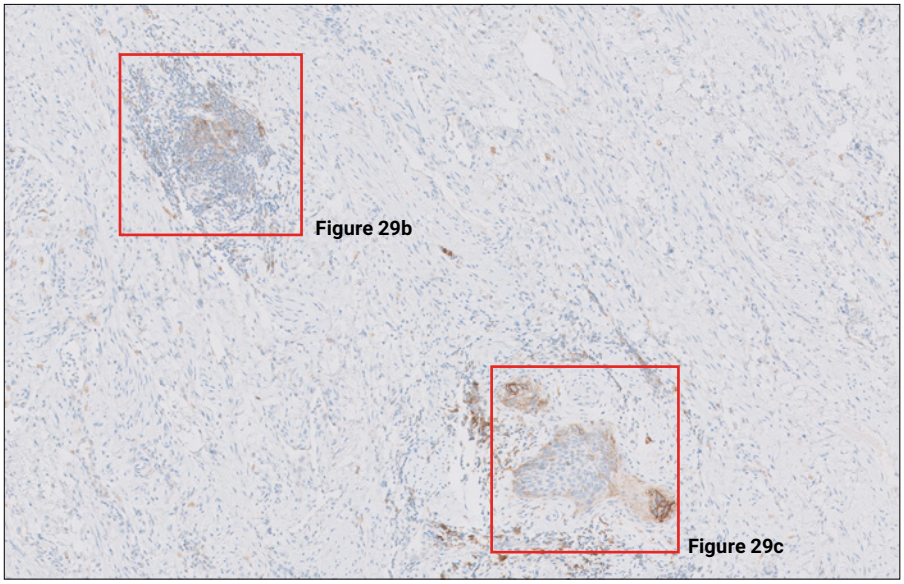


Figure 29a: At 5× magnification, two areas of PD-L1 staining mononuclear inflammatory cells are visible. Following the instructions above, zoom in to 20× magnification on each field to determine which immune cells to include in the numerator (5× magnification).

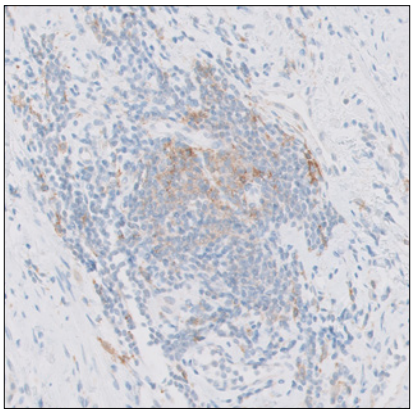


Figure 29b: Tumor cells are absent from this 20× field containing PD-L1 staining mononuclear inflammatory cells, thus none of these cells should be included in the numerator (20× magnification).

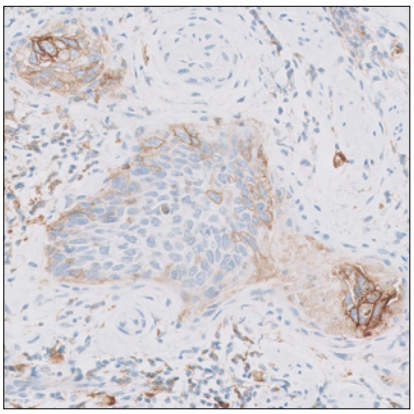


Figure 29c: When positioning the tumor cells in the approximate center of a 20× field, PD-L1 staining mononuclear inflammatory cells that are present within the same field should be included in the numerator (20× magnification).

Cells Excluded from CPS

Only tumor cells exhibiting PD-L1 membrane staining and MICs exhibiting PD-L1 membrane and/or cytoplasmic staining should be included in the CPS numerator. Below are other cells that can exhibit PD-L1 expression but should be excluded from the CPS calculation (CPS numerator or denominator).

Note: Images that follow represent the most common exclusion elements, therefore not all exclusions are represented by images in this manual. Please refer to Tables 1 and 2 on page 26 to view all exclusion criteria.

Tumor Cells with Only Cytoplasmic Staining

Tumor cells exhibiting only cytoplasmic staining are excluded from the CPS numerator. They should, however, still be included in the CPS denominator.

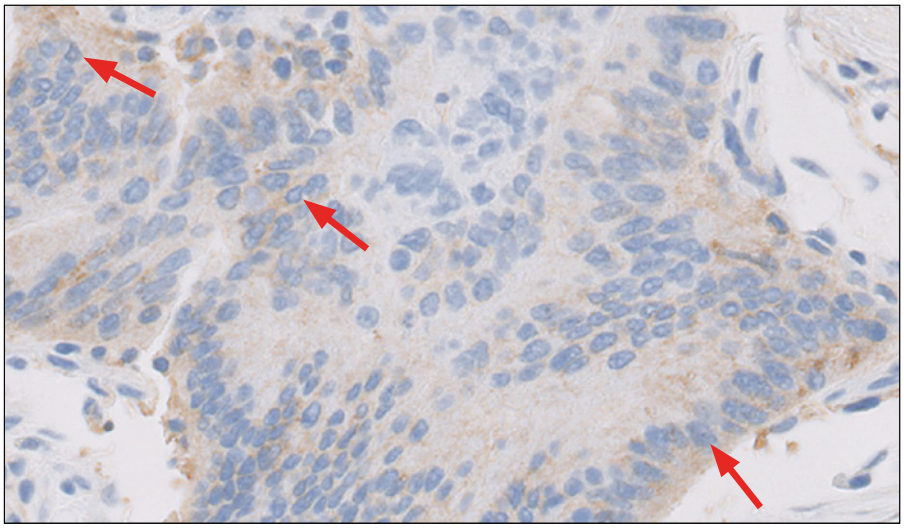


Figure 30: Cervical adenocarcinoma specimen stained with PD-L1 primary antibody exhibiting only cytoplasmic staining of tumor cells (arrows) (20× magnification).

Key point

Tumor cells exhibiting only cytoplasmic staining should not be included in the CPS numerator

Carcinoma in Situ (CIS)

Cervical Intraepithelial Neoplasia (CIN)

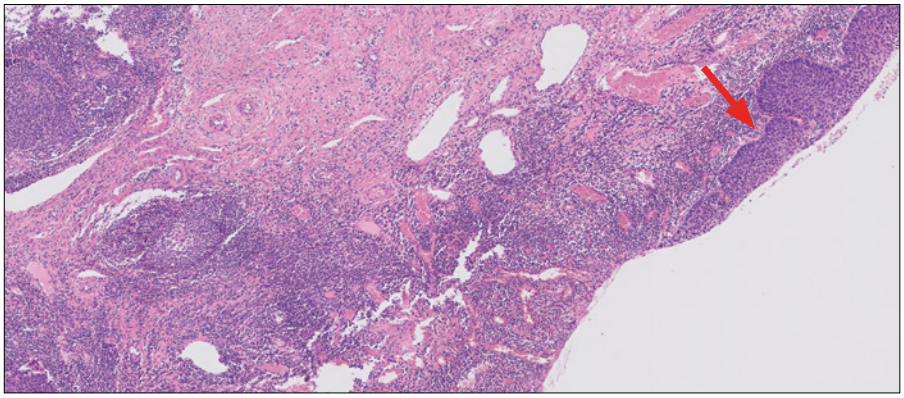


Figure 31a: Hematoxylin and eosin (H&E) section demonstrating cervical intraepithelial neoplasia (CIN) (arrow) (20× magnification).

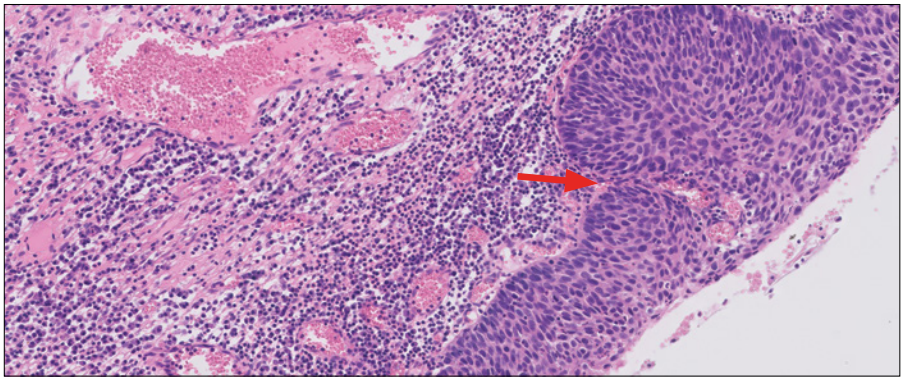


Figure 31b: Hematoxylin and eosin (H&E) section demonstrating cervical intraepithelial neoplasia (CIN) (arrow) (10× magnification).

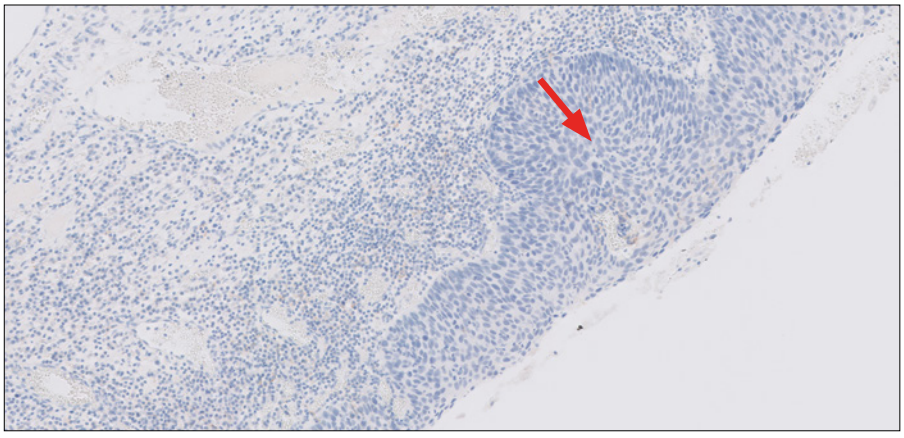


Figure 31c: Any PD-L1 staining of CIN should be excluded from the score (arrow). Non-staining CIN is excluded from the denominator (10× magnification).

Cervical Adenocarcinoma in Situ

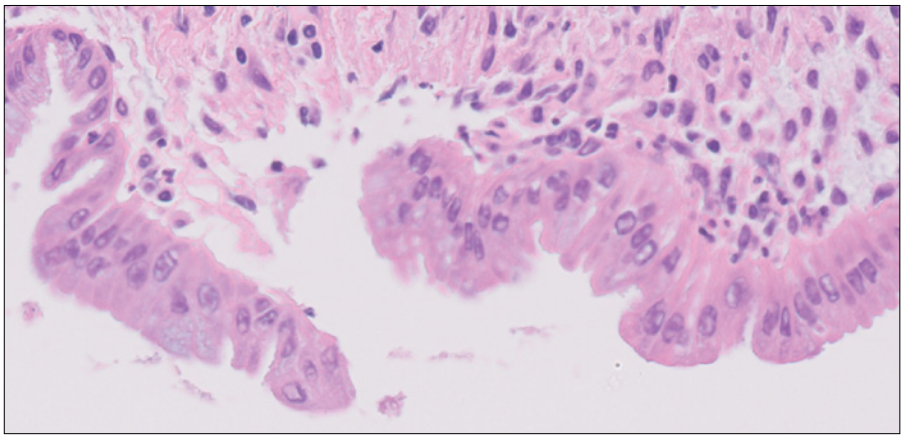


Figure 32a: Hematoxylin and eosin (H&E) section demonstrating cervical adenocarcinoma in situ (20× magnification).

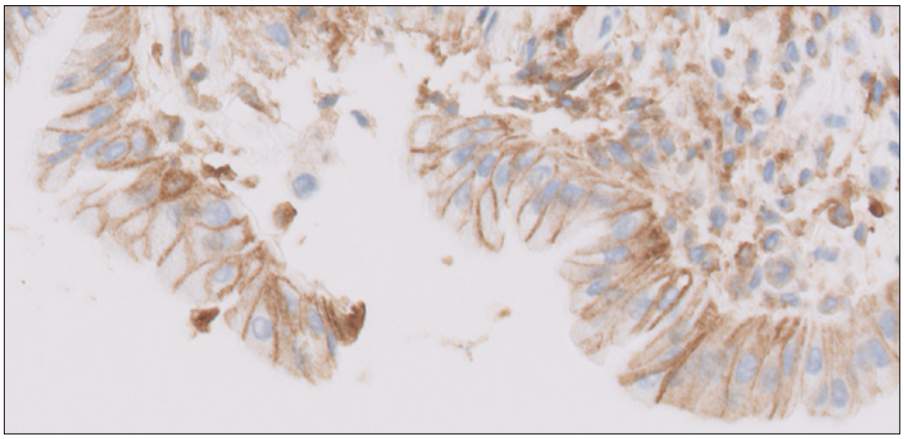


Figure 32b: Cervical adenocarcinoma specimen stained with PD-L1 primary antibody exhibiting cervical adenocarcinoma in situ (20x magnification).

Key point

Cervical carcinoma in situ can exhibit PD-L1 staining and should be excluded from the score

Other Immune Cells Excluded from CPS

Various types of immune cells can exhibit PD-L1 staining, but only tumor-associated lymphocytes and macrophages should be included in the CPS calculation. Refer to page 46 for the immune cell inclusion/exclusion 20× rule. Neutrophils, eosinophils, and plasma cells should be excluded from the CPS calculation.

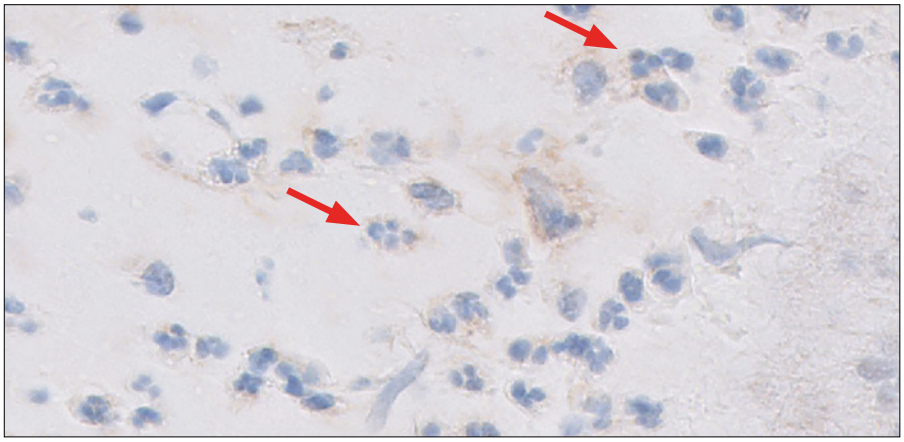


Figure 33a: Cervical squamous cell carcinoma specimen stained with PD-L1 primary antibody exhibiting staining of neutrophils (arrows) (20× magnification).

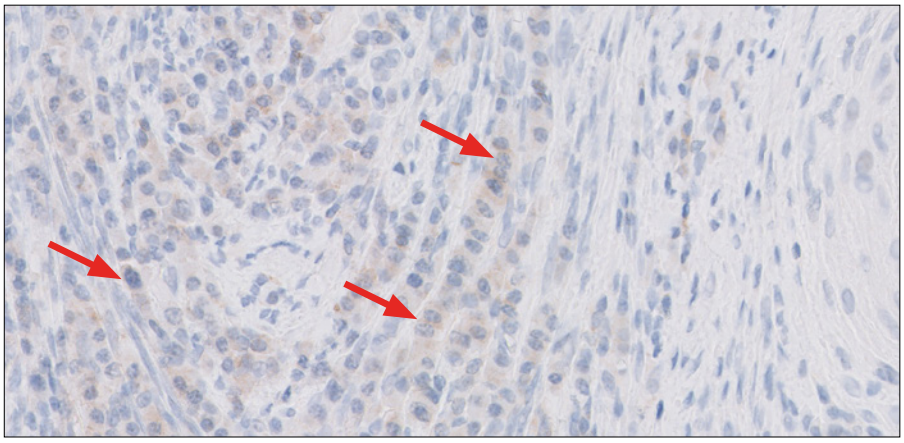


Figure 33b: Cervical squamous cell carcinoma specimen stained with PD-L1 primary antibody exhibiting staining of plasma cells (arrows) (20× magnification).

Key point

Neutrophils, eosinophils, and plasma cells should be excluded from the score

MICs Associated with Benign Cells

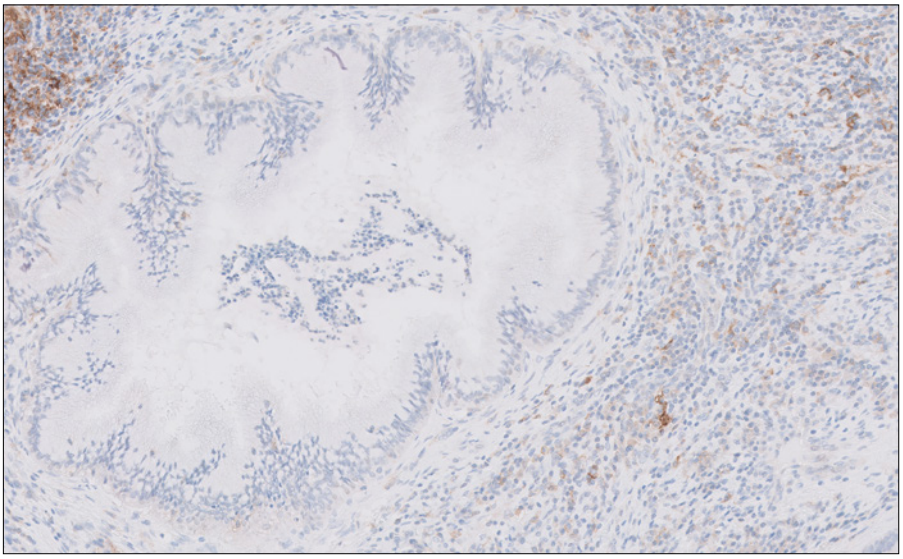


Figure 34: Cervical squamous cell carcinoma specimen stained with PD-L1 primary antibody exhibiting staining of immune cells associated with a benign endocervical gland which should be excluded from the score (10× magnification).

Key point

Both benign cells and immune cells associated with benign cells may exhibit PD-L1 staining and both should be excluded from the score

Stromal Cells

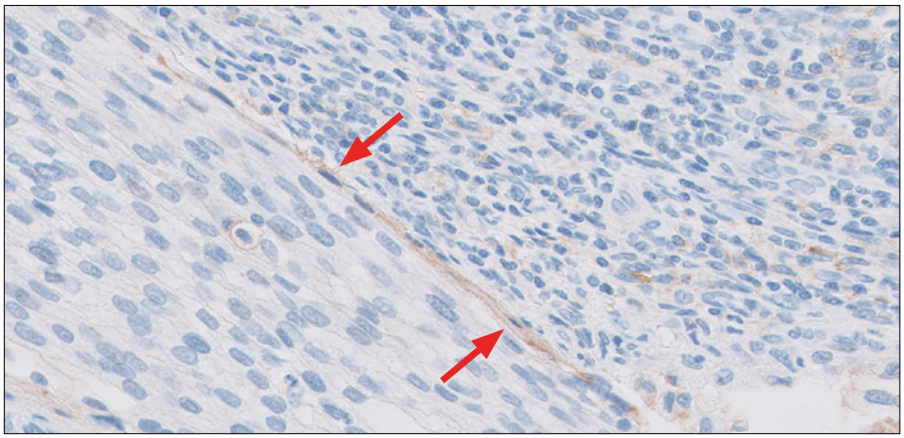


Figure 35: Cervical squamous cell carcinoma specimen stained with PD-L1 primary antibody exhibiting staining of stromal cells (arrows) (20× magnification).

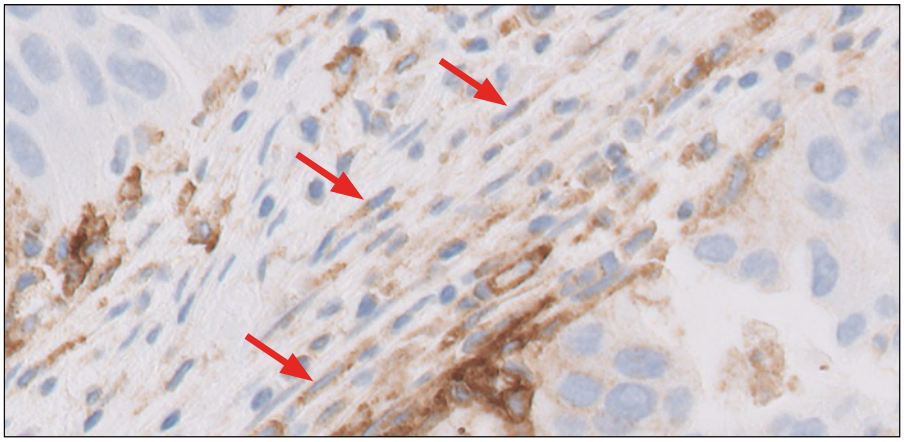


Figure 36: Cervical adenocarcinoma specimen stained with PD-L1 primary antibody exhibiting staining of stromal cells (arrows) (20× magnification).

Key point

PD-L1 staining stromal cells should be excluded from the score

Artifacts

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

Non-specific Staining

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 staining of the NCR-stained test section is useful in determining the level of non-specific staining in the PD-L1 stained test section. All specimens must have ≤ 1+ non-specific staining.

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

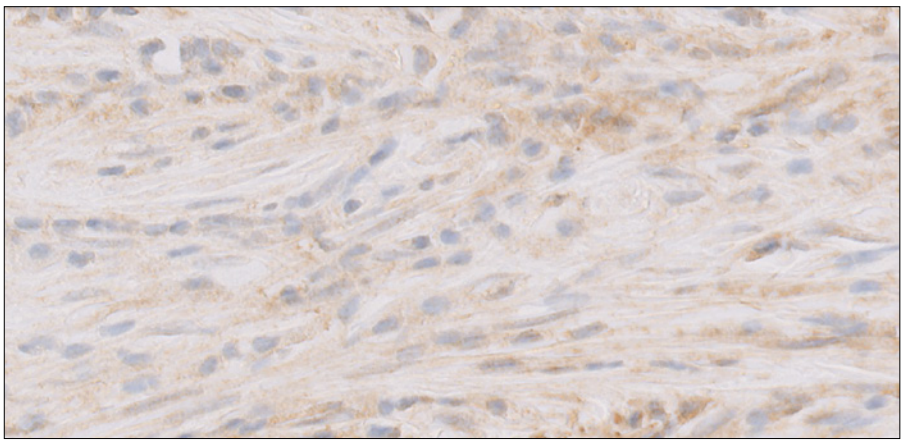


Figure 37a: Cervical adenocarcinoma specimen stained with PD-L1 primary antibody exhibiting non-specific background staining which should be excluded from scoring. Non-specific background staining is defined as diffuse, non-specific staining of a specimen (20× magnification).

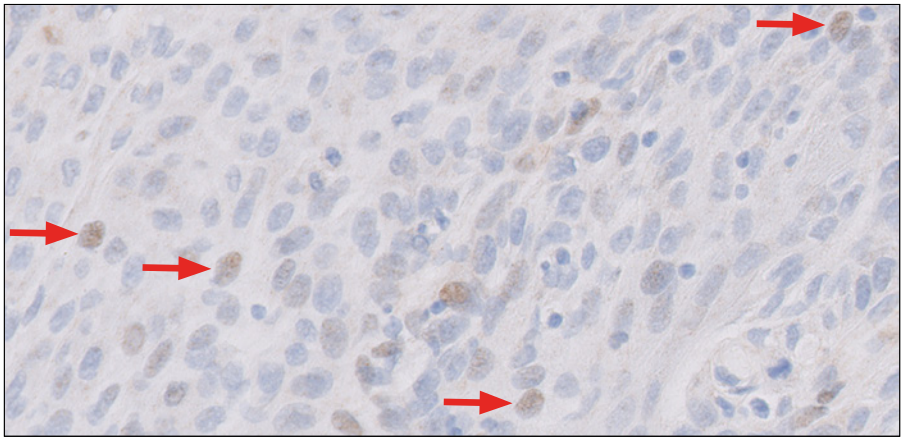


Figure 37b: Cervical squamous cell carcinoma specimen stained with NCR exhibiting non-specific nuclear staining (arrows) (20x magnification).

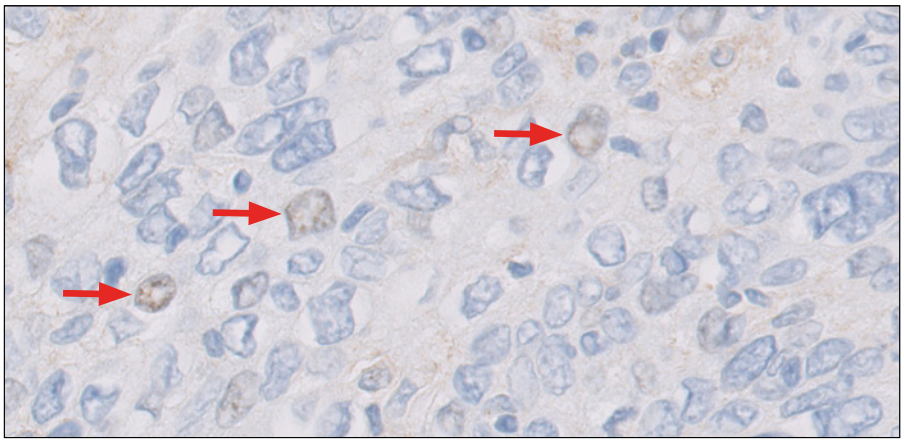


Figure 37c: Cervical squamous cell carcinoma specimen stained with PD-L1 primary antibody exhibiting non-specific nuclear staining (arrows) (20x magnification).

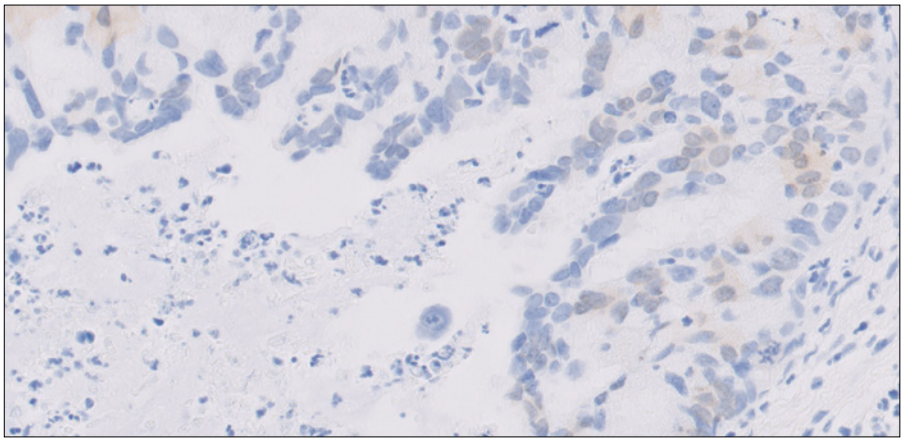


Figure 37d: Cervical adenocarcinoma specimen stained with PD-L1 primary antibody exhibiting non-specific DAB droplets staining which should be excluded from scoring (20x magnification).

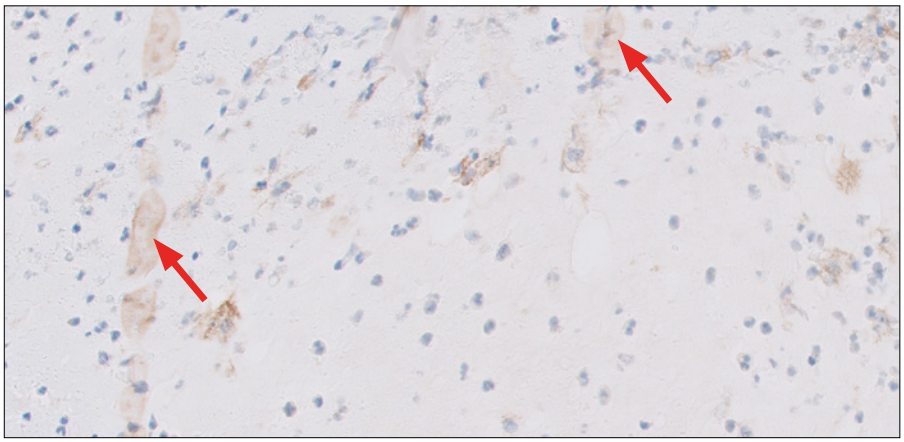


Figure 37e: Cervical squamous cell carcinoma specimen stained with PD-L1 primary antibody exhibiting non-specific DAB staining (arrows) which should be excluded from the score (20× magnification).

Key point

All specimens must have ≤ 1+ non-specific 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. In this case, only PD-L1 staining at the edge of the tissue section is excluded from scoring.

Note: Although edge artifact can be present, it is not as commonly seen as in other IHC stains.

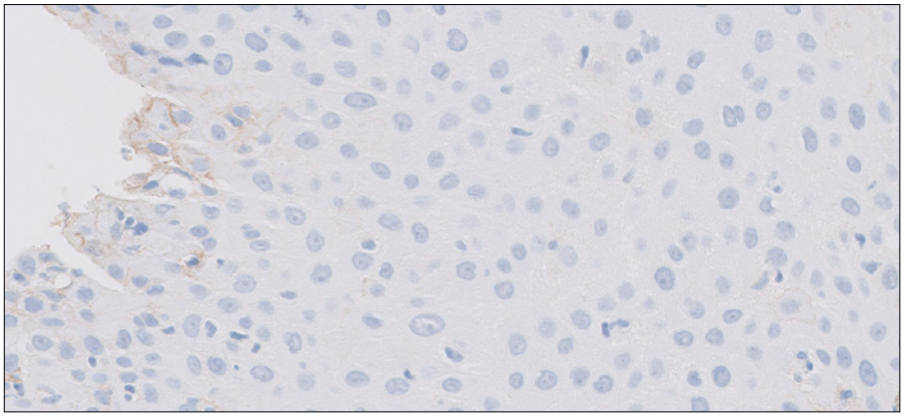


Figure 38: Cervical squamous cell carcinoma specimen stained with PD-L1 primary antibody exhibiting edge staining which should be excluded from the score (20× magnification).

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.

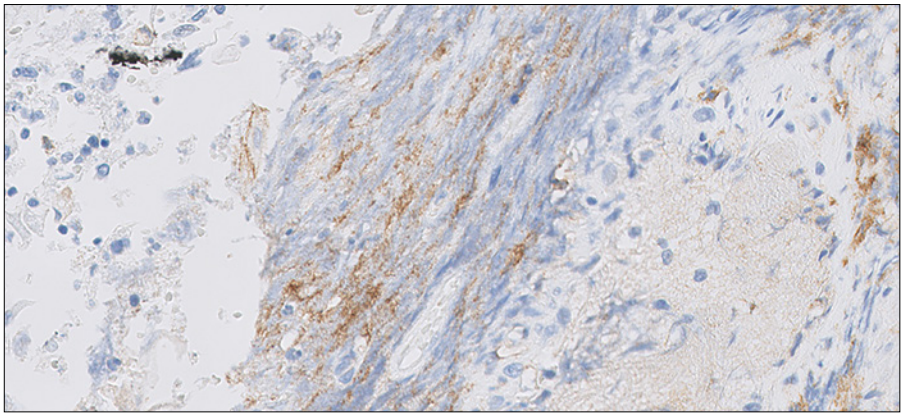


Figure 39: Esophageal cancer specimen stained with PD-L1 primary antibody exhibiting crush artifact; crush artifact should be excluded from the score (20× magnification).

Key Point

Scoring of crush artifact should be avoided

Poor Fixation

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

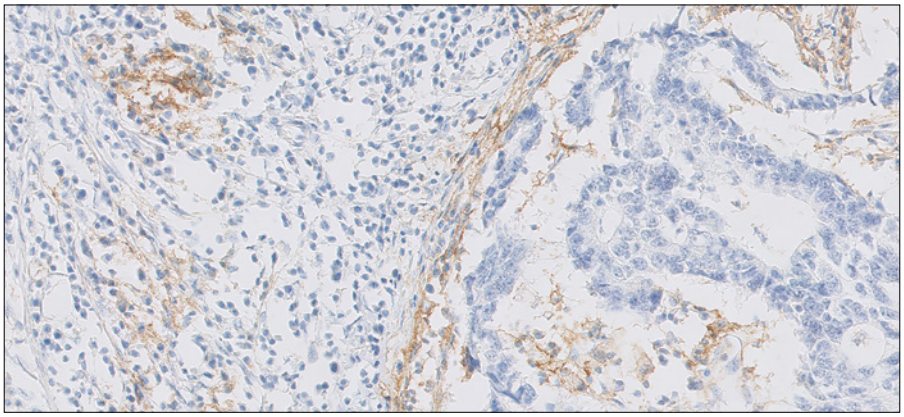


Figure 40: Esophageal cancer specimen stained with PD-L1 primary antibody exhibiting poor tissue fixation (20× magnification).

Key Point

Proper fixation is important for accurate PD-L1 assessment

Necrosis

Necrosis can be described as morphological changes indicative of cell death with undefined cellular detail. PD-L1 staining necrosis is often present in cervical cancer specimens and should be excluded from the score.

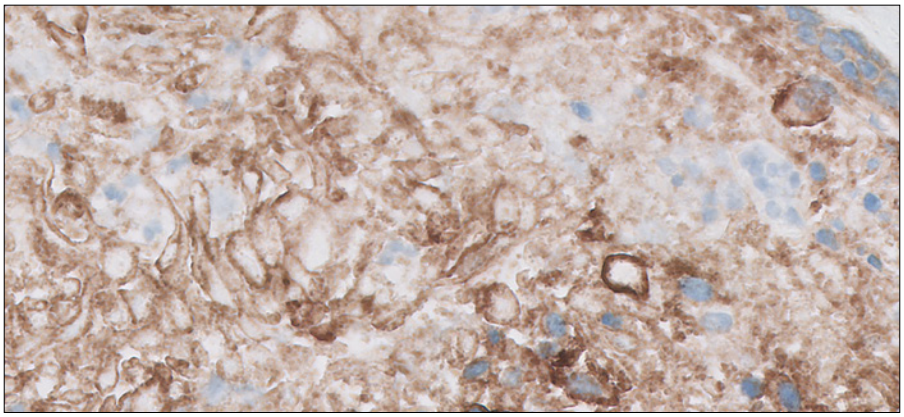


Figure 41: Cervical squamous cell carcinoma specimen stained with PD-L1 primary antibody exhibiting staining of necrosis which should be excluded from the score (20× magnification).

Key Point

PD-L1 staining necrosis should not be scored

PD-L1 IHC 22C3 pharmDx CPS Case Examples

CPS 0 Case Example

Case 1: CPS 0

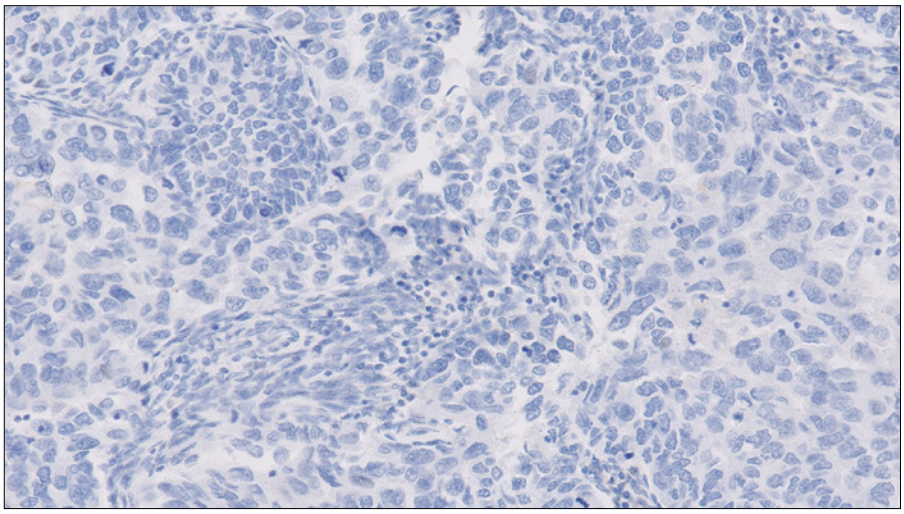


Figure 42: Cervical adenocarcinoma specimen stained with PD-L1 antibody exhibiting a CPS of 0 (20× magnification).

CPS ≥ 1 Case Examples

Case 2: CPS ≥ 1

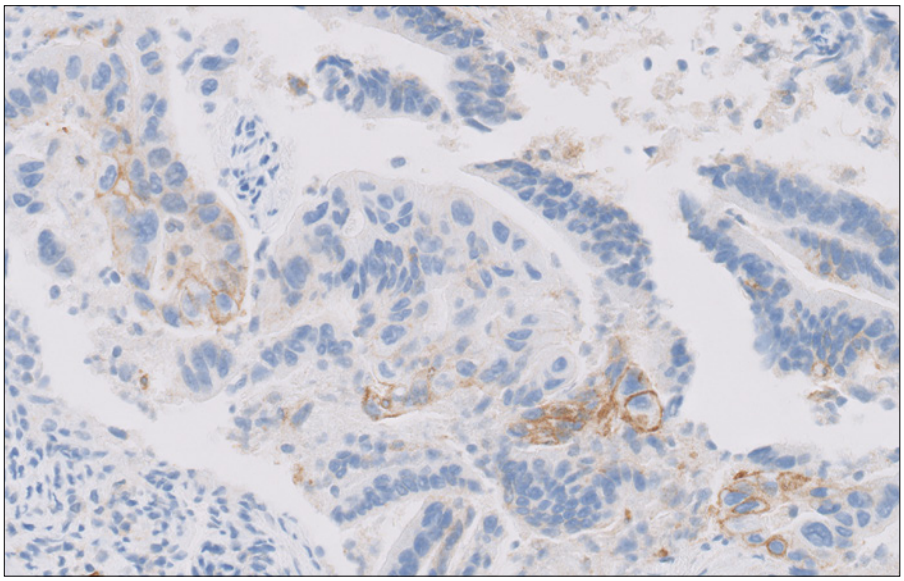


Figure 43: Cervical adenocarcinoma specimen stained with PD-L1 antibody exhibiting a CPS of 12, however any numerical CPS between 10–14 could be assigned to this image (20× magnification).

Case 3: CPS ≥ 1

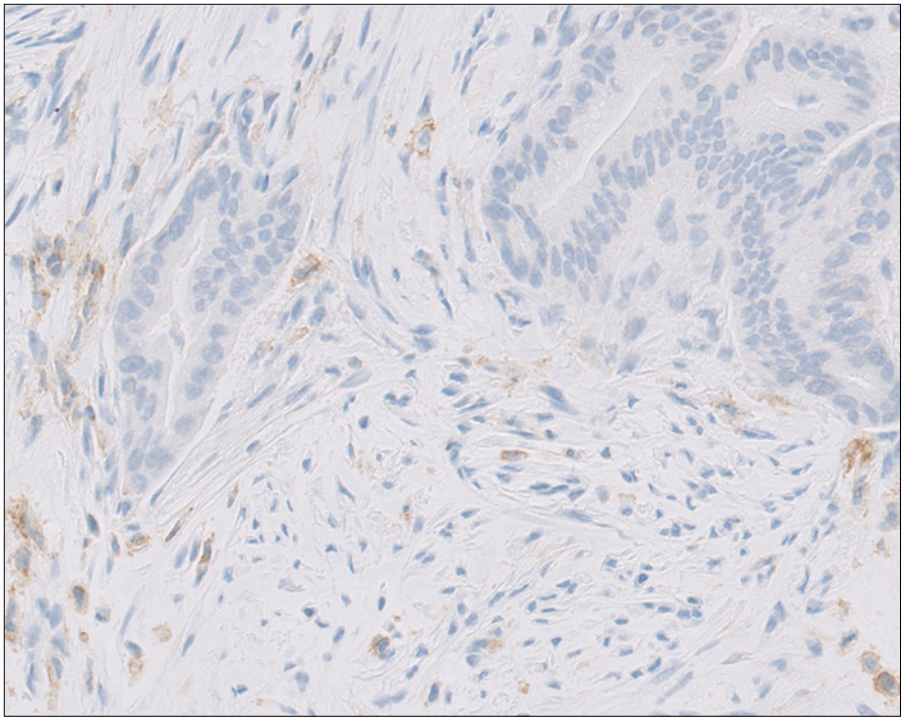


Figure 44: Cervical adenocarcinoma specimen stained with PD-L1 antibody exhibiting a CPS of 12, however any numerical CPS between 10–14 could be assigned to this image (20× magnification).

Case 4: CPS ≥ 1

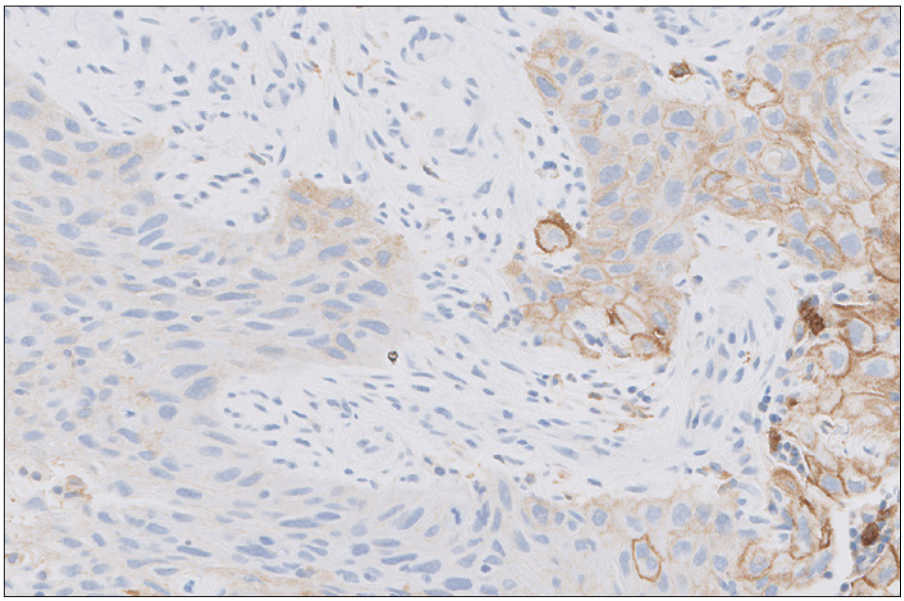


Figure 45: Cervical squamous cell carcinoma specimen stained with PD-L1 antibody exhibiting a CPS of 31, however any numerical CPS between 28–34 could be assigned to this image (20× magnification).

Case 5: CPS ≥ 1

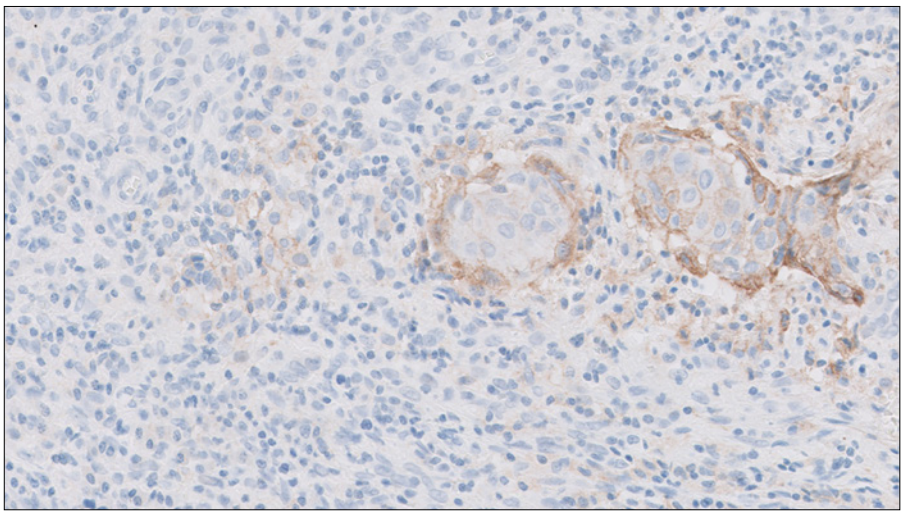


Figure 46: Cervical squamous cell carcinoma specimen stained with PD-L1 antibody with borderline specimen adequacy due to having close to 100 viable invasive tumor cells. This specimen is exhibiting a CPS of 60, however any numerical CPS between 57–63 could be assigned to this image (20× magnification)

Case 6: CPS ≥ 1

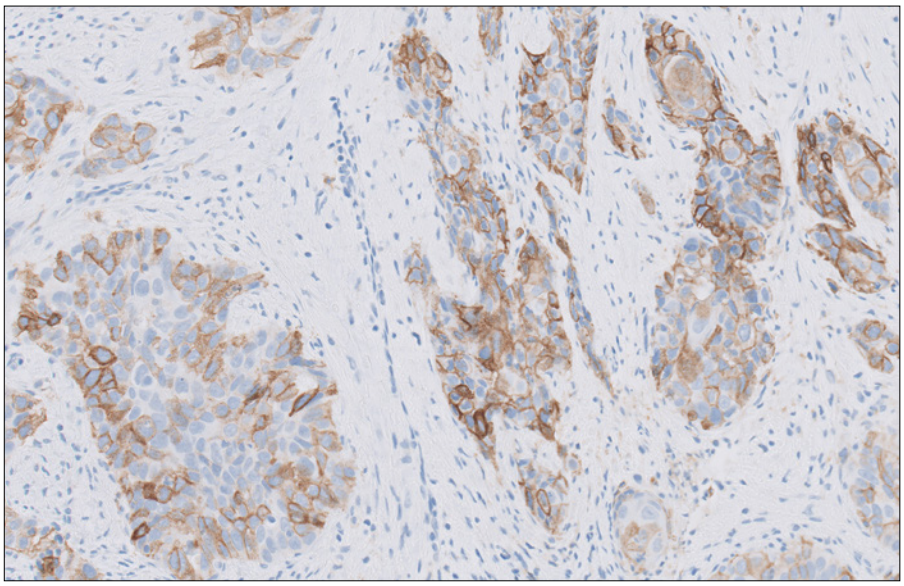


Figure 47: Cervical squamous cell carcinoma specimen stained with PD-L1 antibody exhibiting a CPS of 73, however any numerical CPS between 70–76 could be assigned to this image (20× magnification).

Case 7: CPS ≥ 1

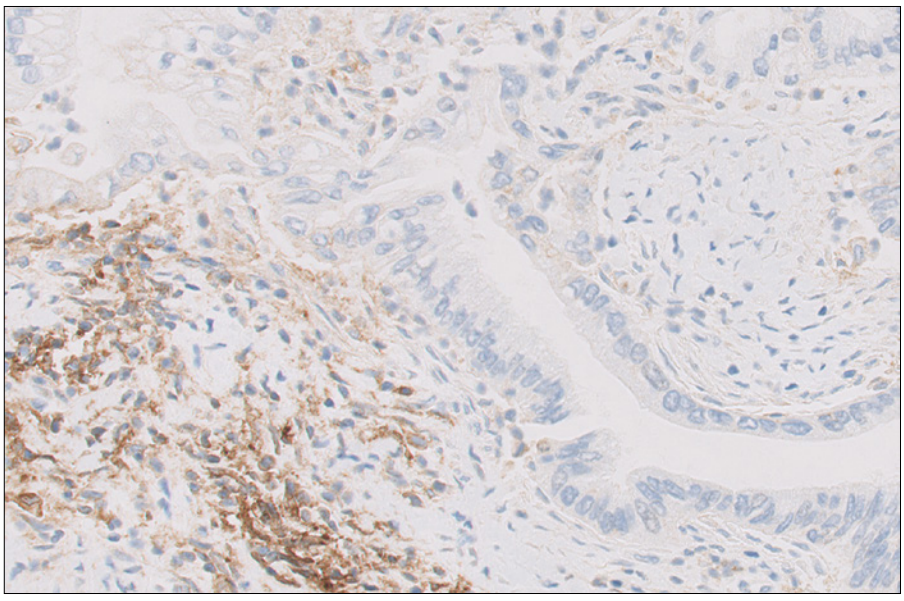


Figure 48: Cervical adenocarcinoma specimen stained with PD-L1 antibody exhibiting a CPS of 74, however any numerical CPS between 71–77 could be assigned to this image (20× magnification).

Case 8: CPS ≥ 1

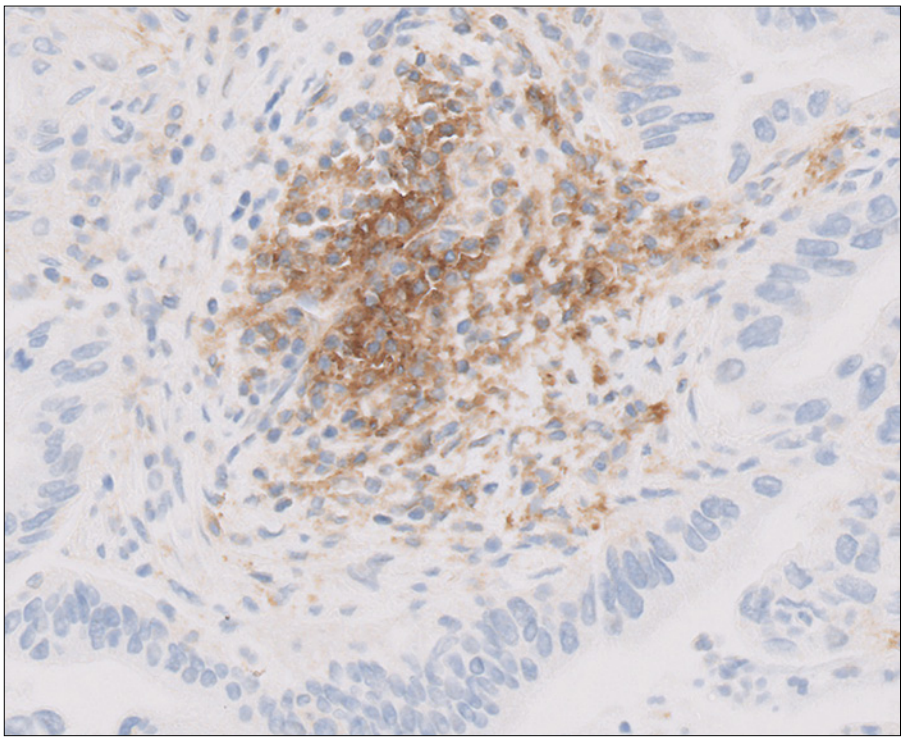


Figure 49: Cervical adenocarcinoma specimen stained with PD-L1 antibody exhibiting a CPS of 90, however any numerical CPS between 87–93 could be assigned to this image (20× magnification).

Case 9: CPS ≥ 1

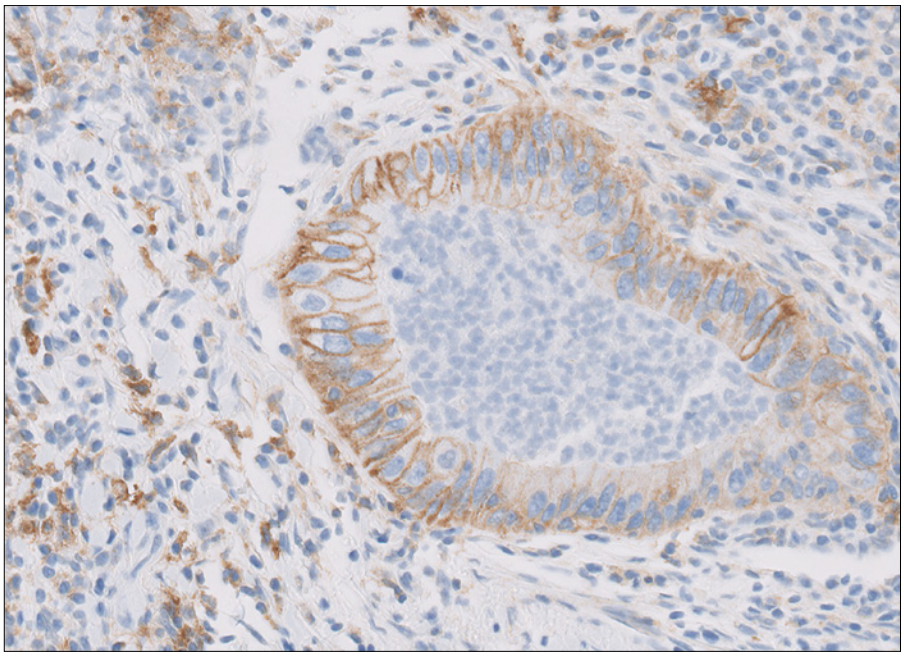


Figure 50: Cervical adenocarcinoma specimen stained with PD-L1 antibody exhibiting a CPS of 98, however any numerical CPS between 95–100 could be assigned to this image (20× magnification).

Case 10: CPS ≥ 1

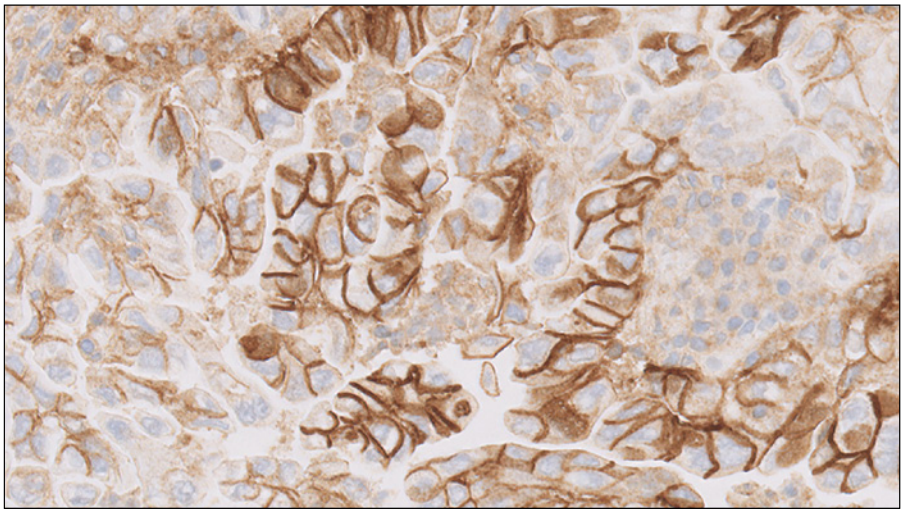


Figure 51: Cervical adenocarcinoma specimen stained with PD-L1 antibody exhibiting a CPS of 100 (20× magnification).

Case 11: CPS ≥ 1

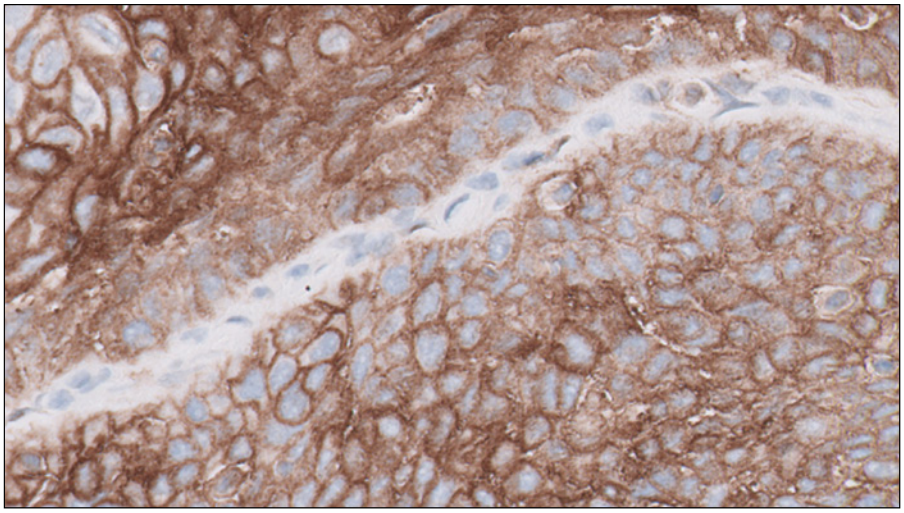


Figure 52: Cervical squamous cell carcinoma specimen stained with PD-L1 antibody exhibiting a CPS of 100 (20× magnification).

Near-Cutoff Case Examples (CPS Range of Greater Than 0 but Less Than or Equal to 10)

Challenging Case 1: Near-Cutoff (CPS Range of Greater Than 0 but Less Than or Equal to 10)

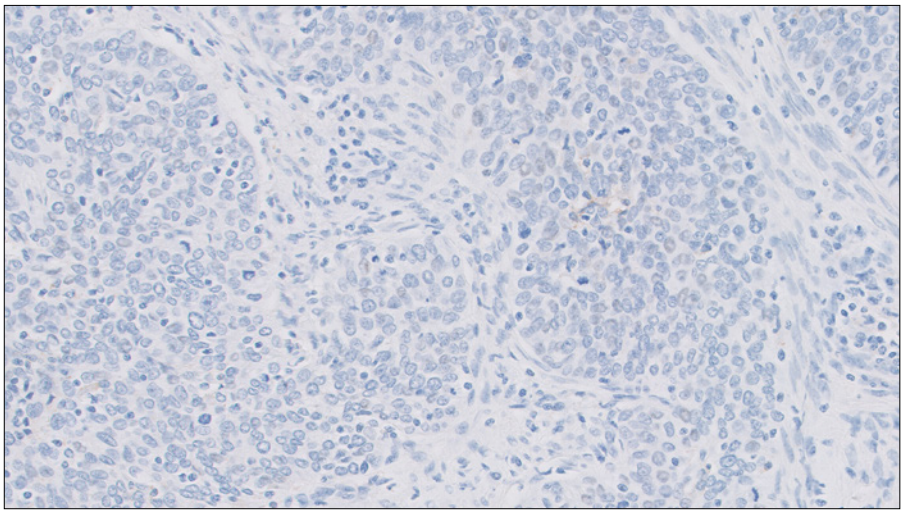


Figure 53: Cervical squamous cell carcinoma specimen stained with PD-L1 antibody exhibiting a CPS < 1 (20× magnification).

Challenging Case 2: Near-Cutoff (CPS Range of Greater Than 0 but Less Than or Equal to 10)

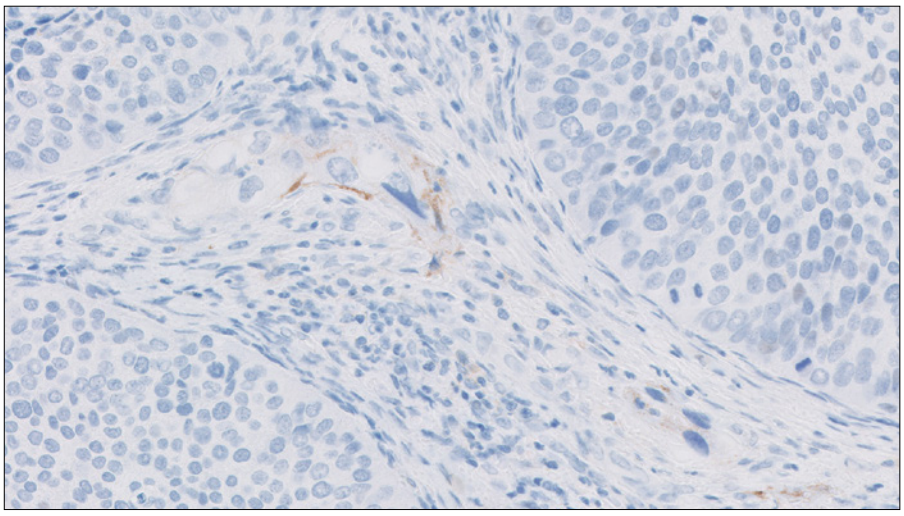


Figure 54: Cervical squamous cell carcinoma specimen stained with PD-L1 antibody exhibiting a CPS < 1 (20× magnification).

Challenging Case 3: Near-Cutoff (CPS Range of Greater Than 0 but Less Than or Equal to 10)

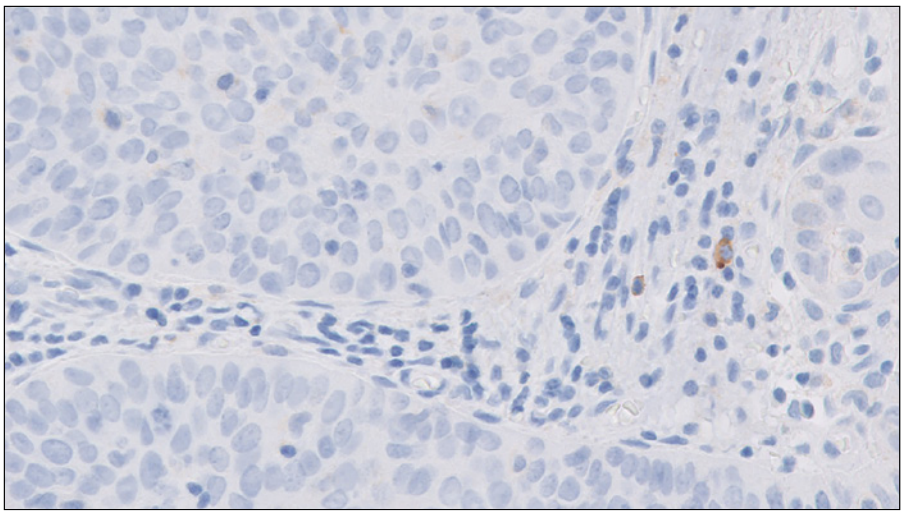


Figure 55: Cervical squamous cell carcinoma specimen stained with PD-L1 antibody exhibiting a CPS < 1 (20× magnification).

Challenging Case 4: Near-Cutoff (CPS Range of Greater Than 0 but Less Than or Equal to 10)

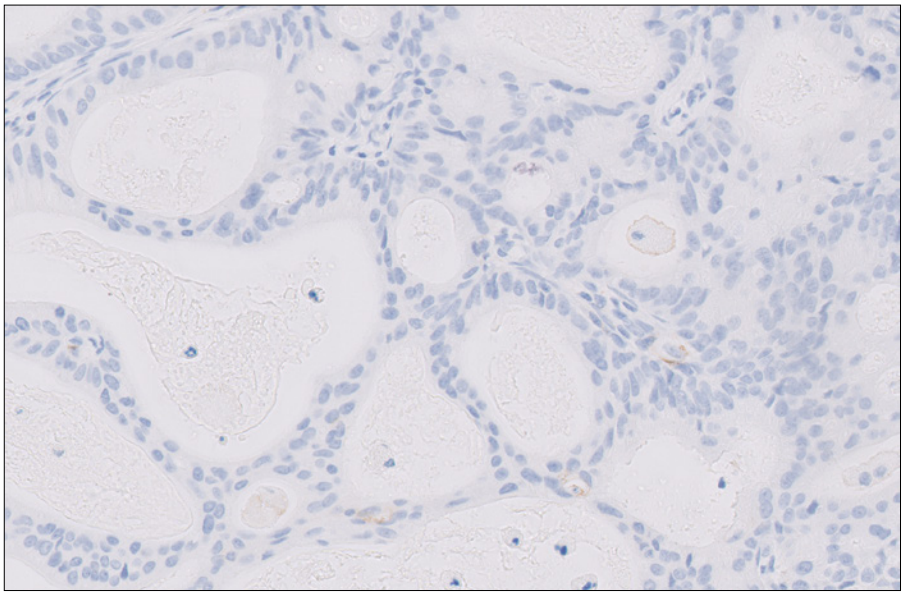


Figure 56: Cervical adenocarcinoma specimen stained with PD-L1 antibody exhibiting a CPS < 1 (20× magnification).

Challenging Case 5: Near-Cutoff (CPS Range of Greater Than 0 but Less Than or Equal to 10)

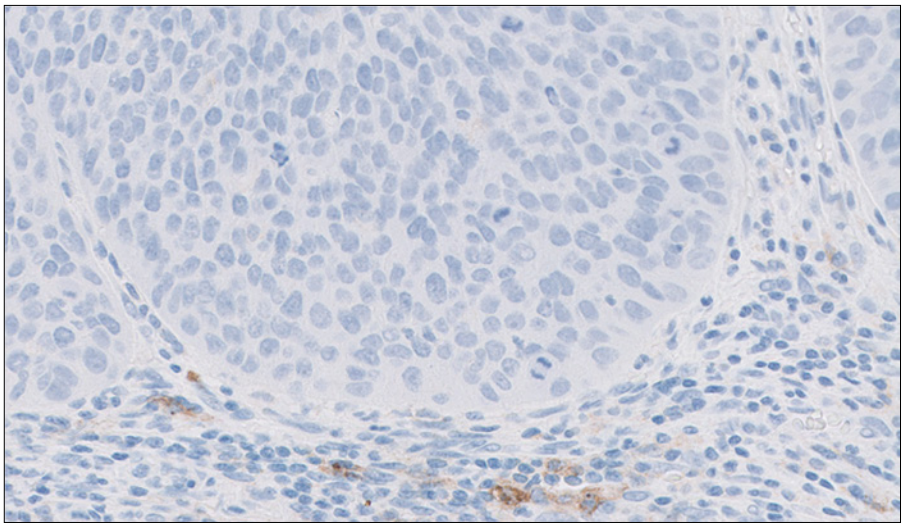


Figure 57: Cervical squamous cell carcinoma specimen stained with PD-L1 antibody exhibiting a CPS of 1, however any numerical CPS between 1–2 could be assigned to this image (20× magnification).

Challenging Case 6: Near-Cutoff (CPS Range of Greater Than 0 but Less Than or Equal to 10)

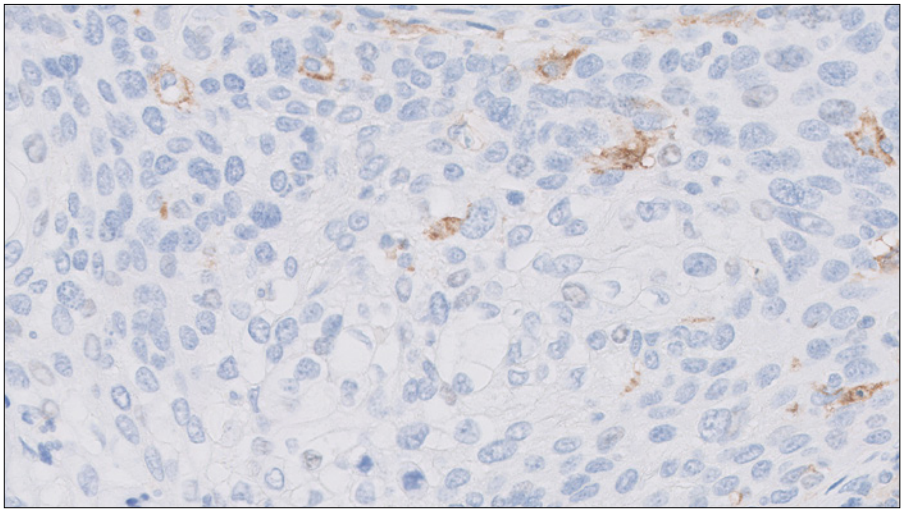


Figure 58: Cervical squamous cell carcinoma specimen stained with PD-L1 antibody exhibiting a CPS of 2, however any numerical CPS between 1–3 could be assigned to this image (20× magnification).

Challenging Case 7: Near-Cutoff (CPS Range of Greater Than 0 but Less Than or Equal to 10)

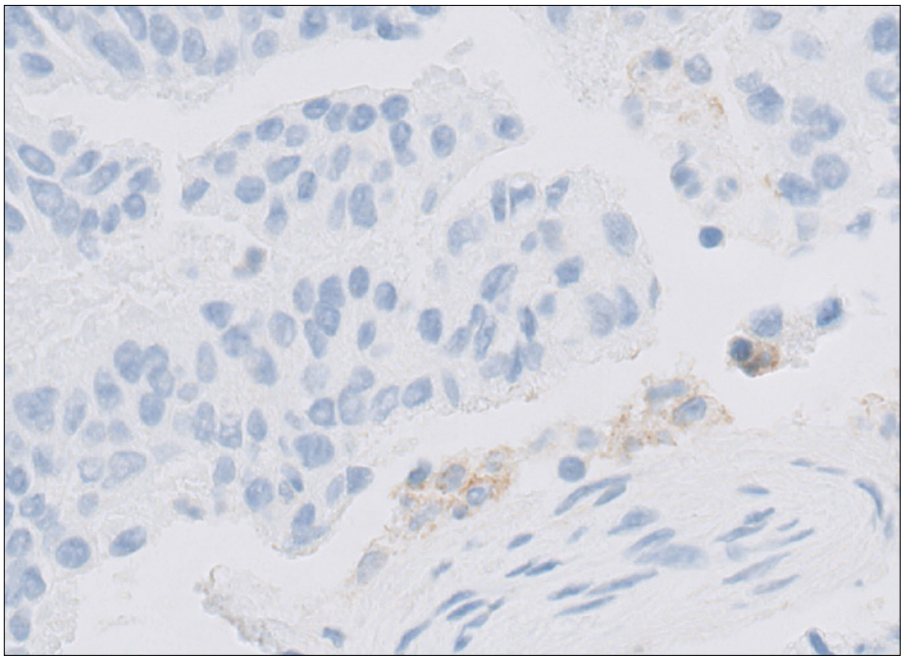


Figure 59: Cervical adenocarcinoma specimen stained with PD-L1 antibody exhibiting a CPS of 6, however any numerical CPS between 4–8 could be assigned to this image (20× magnification).

Challenging Case 8: Near-Cutoff (CPS Range of Greater Than 0 but Less Than or Equal to 10)

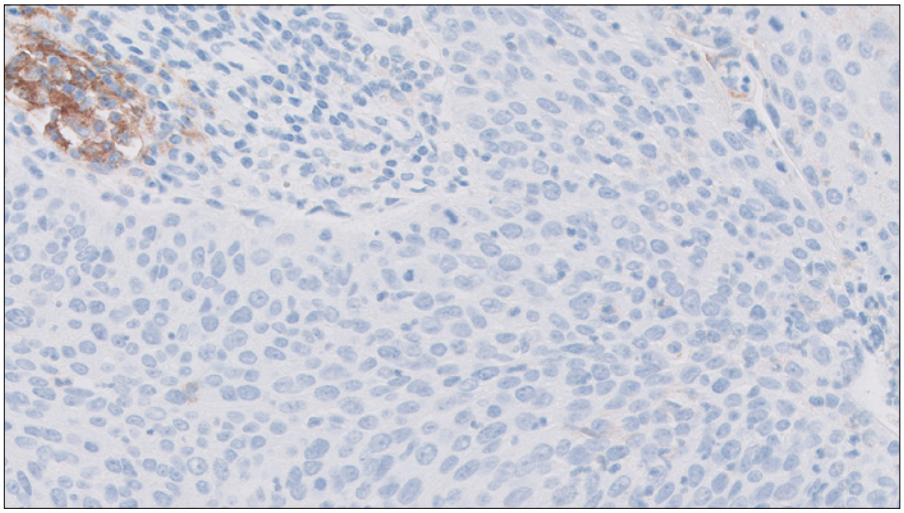


Figure 60: Cervical squamous cell carcinoma specimen stained with PD-L1 antibody exhibiting a CPS of 6, however any numerical CPS between 4–8 could be assigned to this image (20× magnification).

Challenging Case 9: Near-Cutoff (CPS Range of Greater Than 0 but Less Than or Equal to 10)

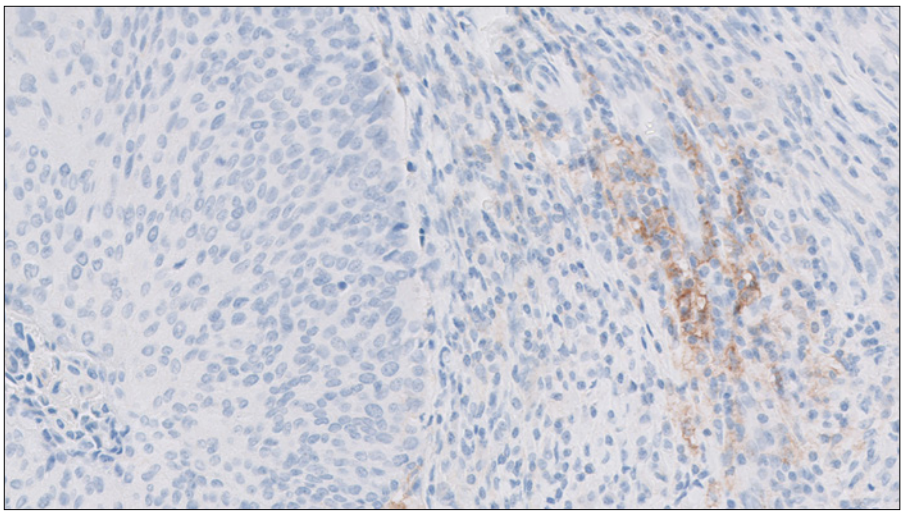


Figure 61: Cervical squamous cell carcinoma specimen stained with PD-L1 antibody exhibiting a CPS of 8, however any numerical CPS between 6–10 could be assigned to this image (20× magnification).

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

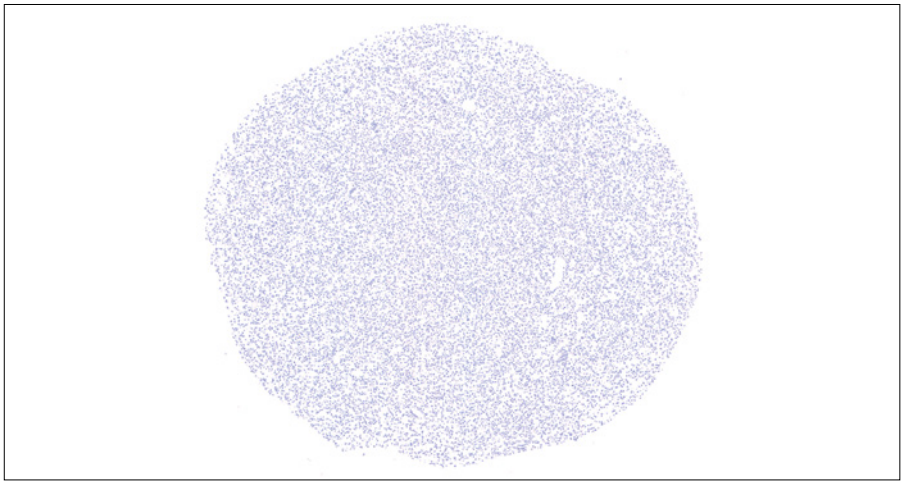


Figure 62: MCF-7 cell pellet (2× magnification).

Passing PD-L1 Positive CCL

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

Figure 63: NCI-H226 cell pellet (2× magnification).

Borderline Passing CCL

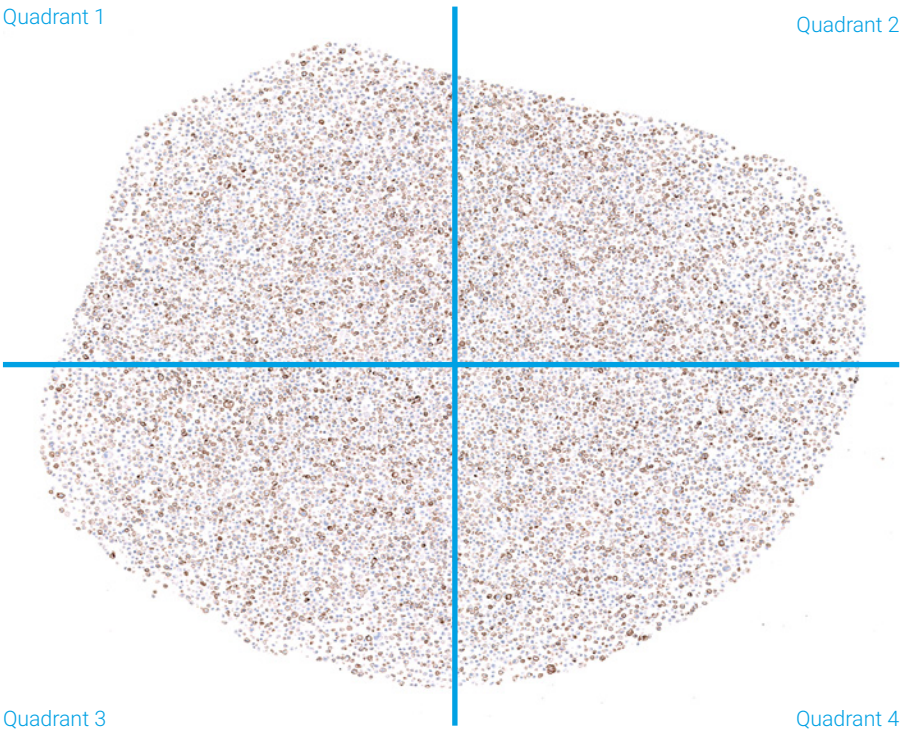
Borderline Passing vs. Passing PD-L1 Positive CCL

Borderline Passing PD-L1 positive CCL

Figure 64: 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+.

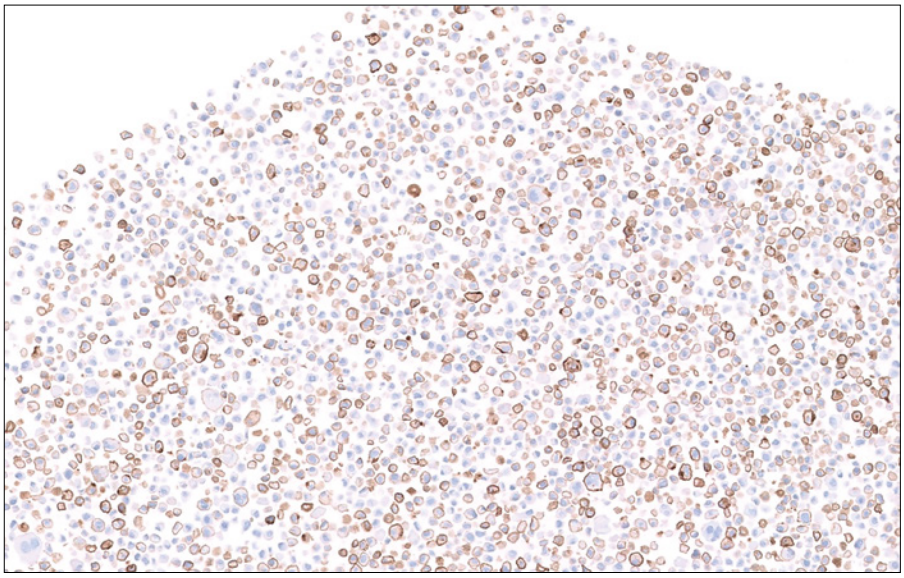


Figure 65: NCI-H226 cell pellet (5× magnification).

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+.

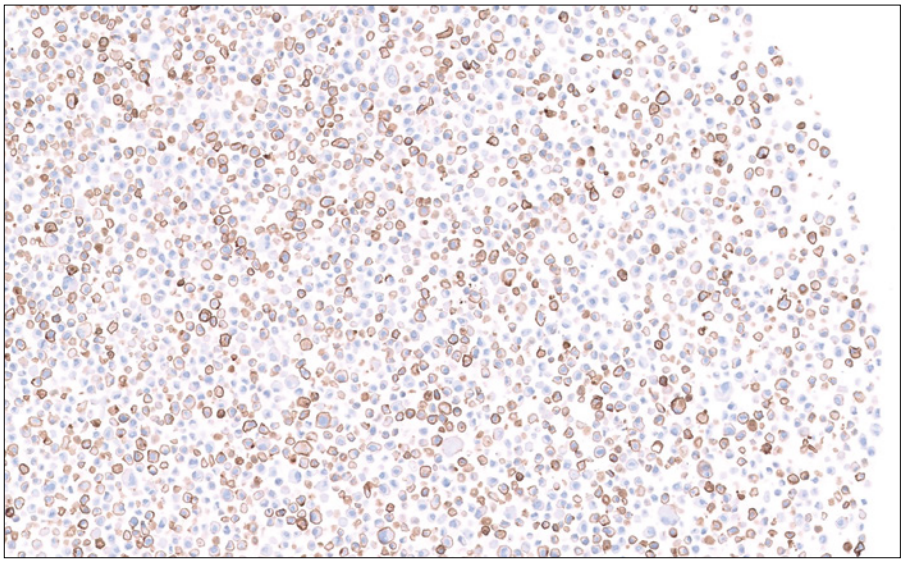


Figure 66: NCI-H226 cell pellet (5× magnification).

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+.

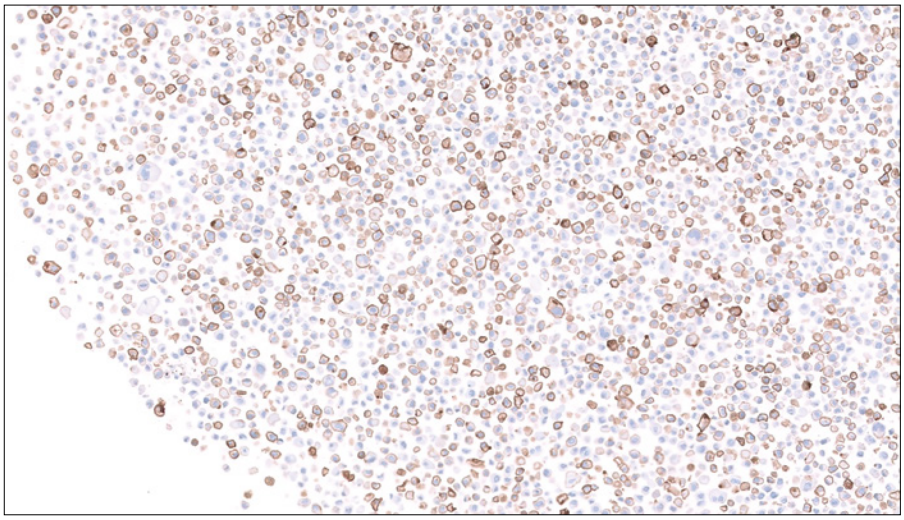


Figure 67: NCI-H226 cell pellet (5× magnification).

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+.

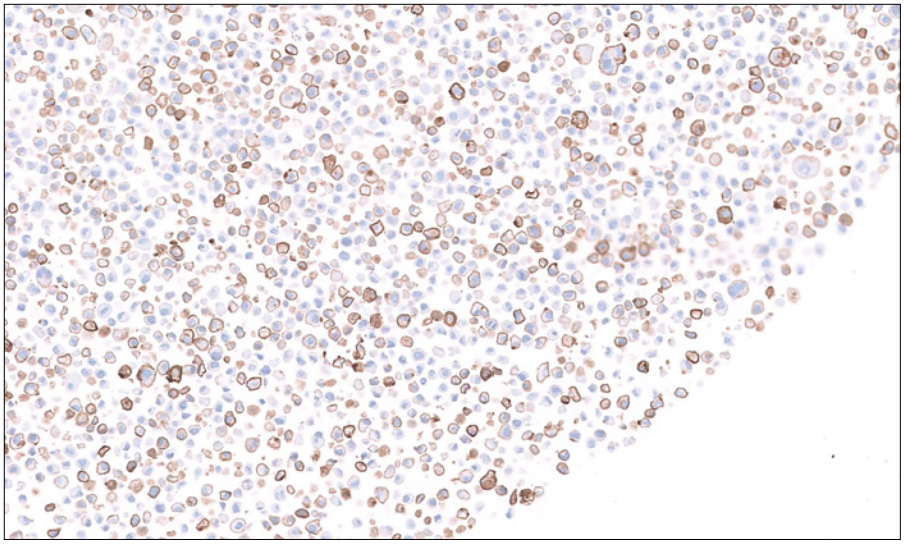
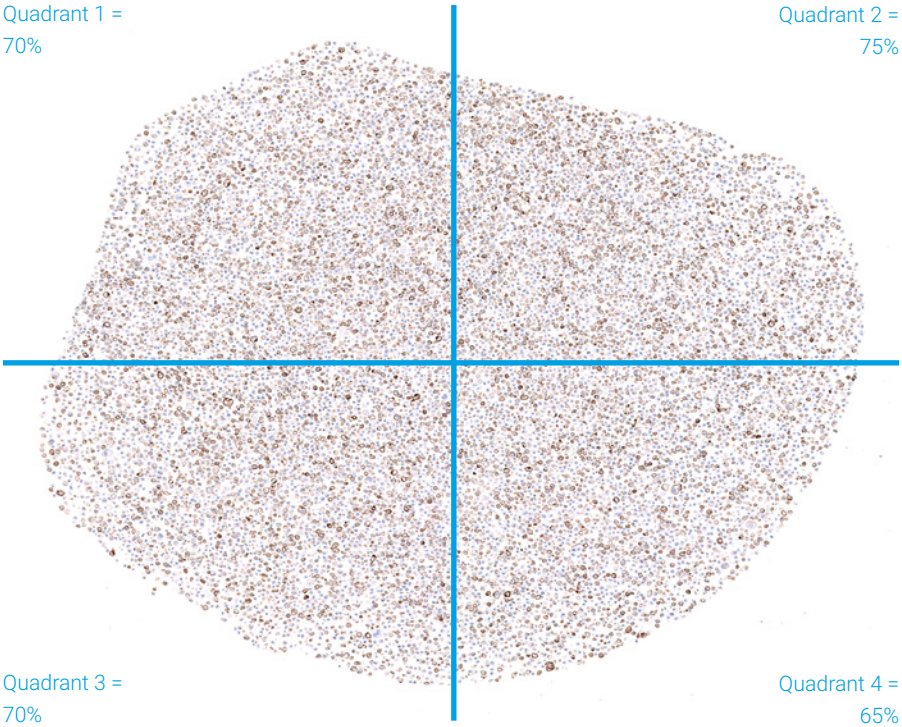


Figure 68: NCI-H226 cell pellet (5× magnification).

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

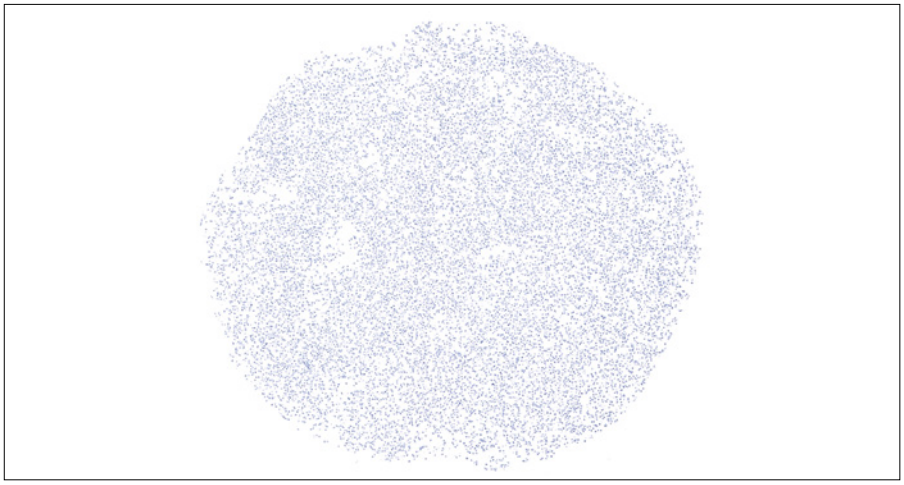


Figure 69: MCF-7 cell pellet (2× magnification).

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+

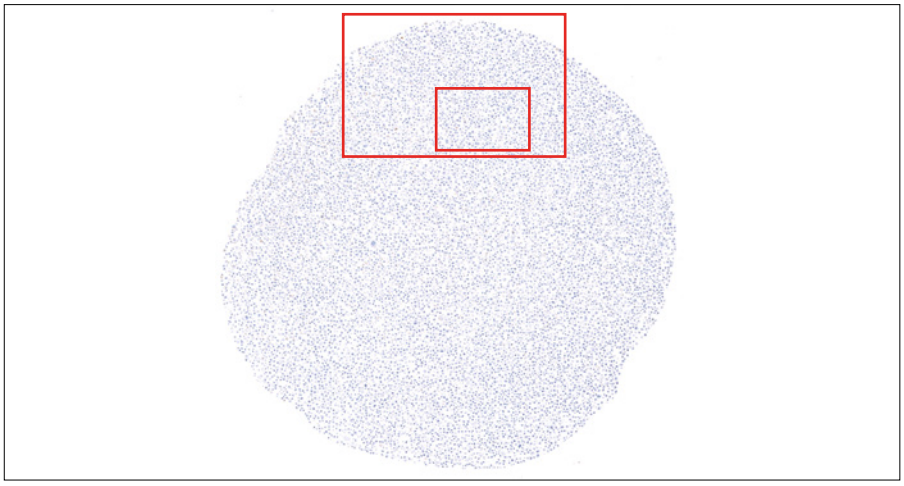


Figure 70: NCI-H226 cell pellet (2× magnification).

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

Failed PD-L1 positive CCL (10×)

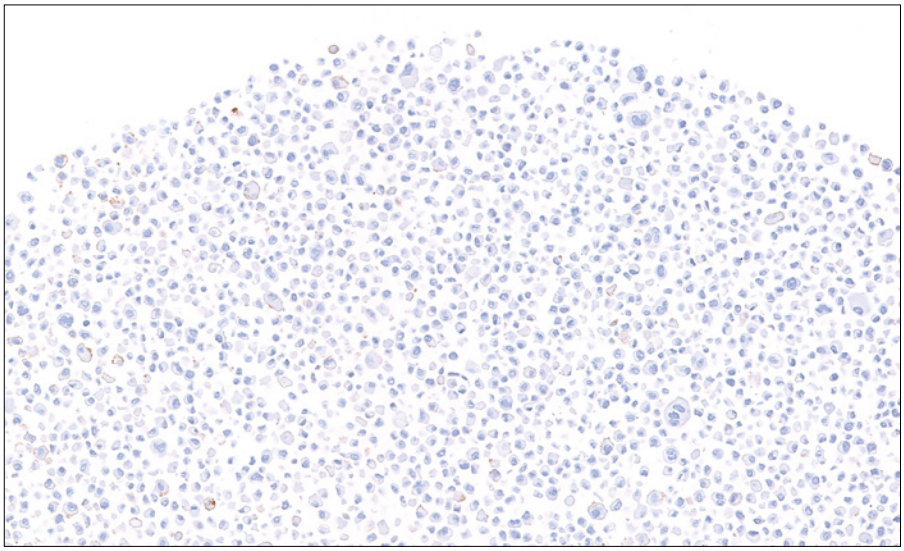


Figure 71: NCI-H226 cell pellet (10× magnification).

Failed PD-L1 positive CCL (20×)

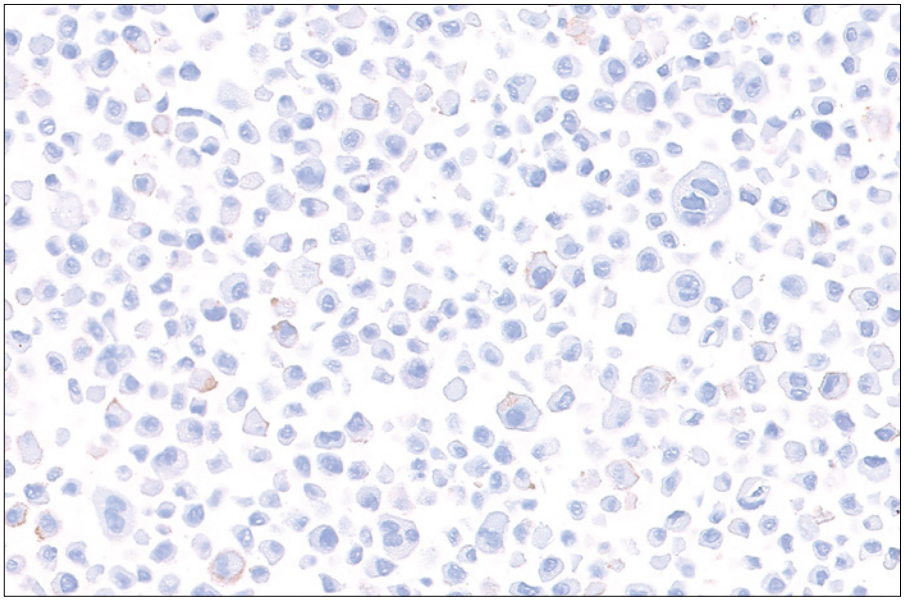


Figure 72: NCI-H226 cell pellet (20× magnification).

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

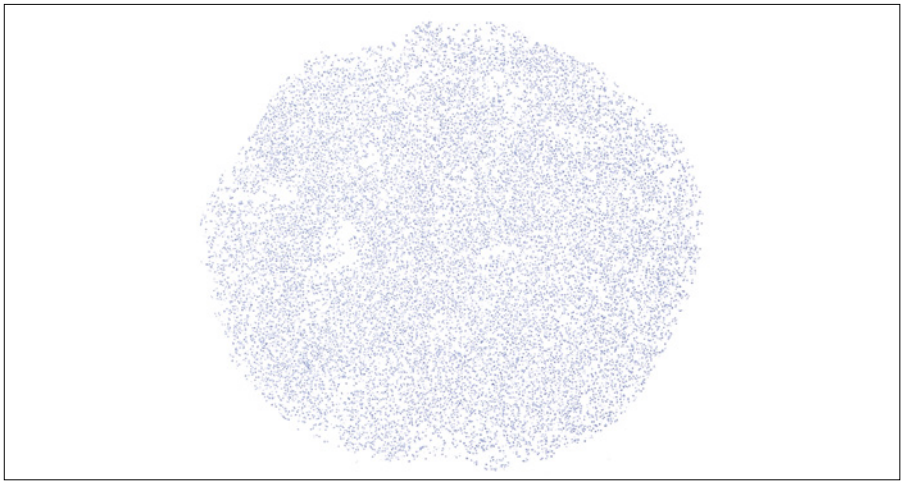


Figure 73: MCF-7 cell pellet (2× magnification).

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

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

Failed PD-L1 positive CCL (10×)

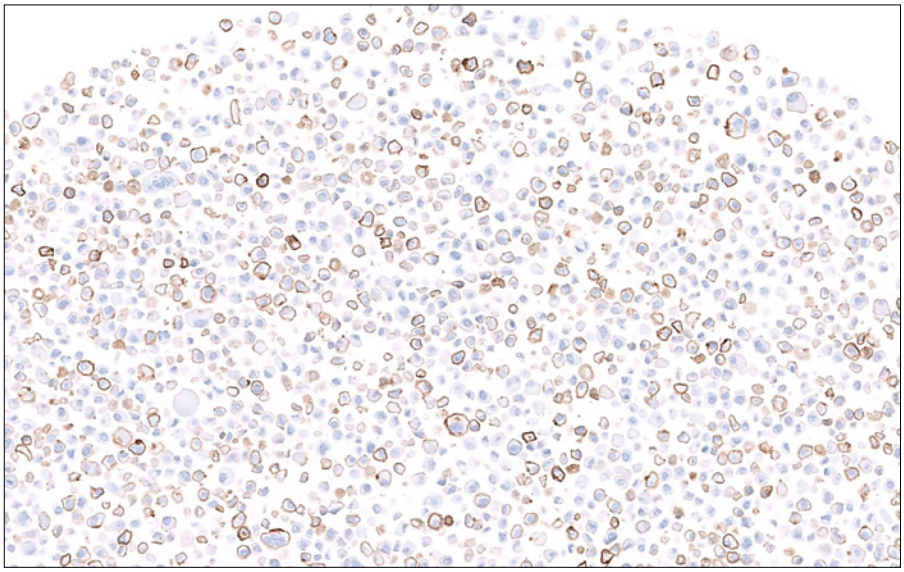


Figure 75: NCI-H226 cell pellet (10× magnification).

Failed PD-L1 positive CCL (20×)

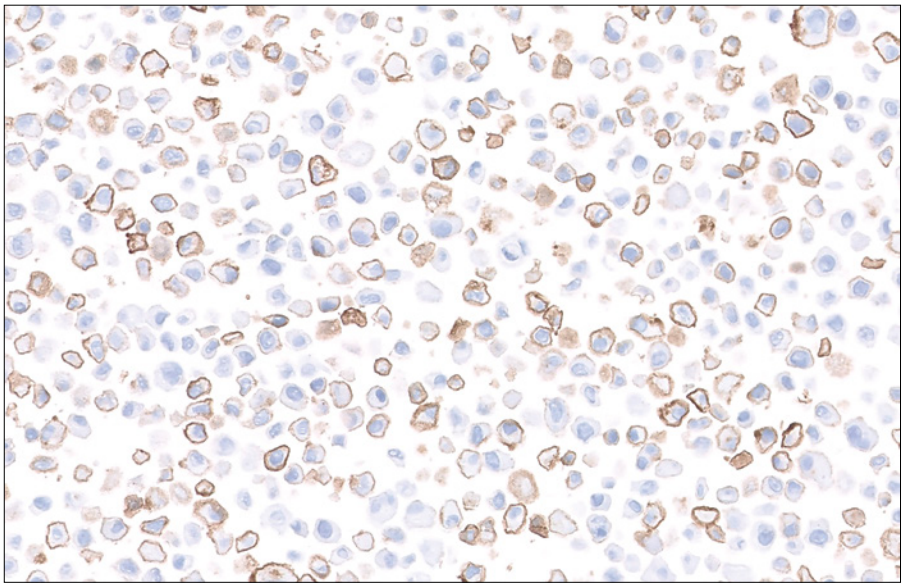


Figure 76: NCI-H226 cell pellet (20× magnification).

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+

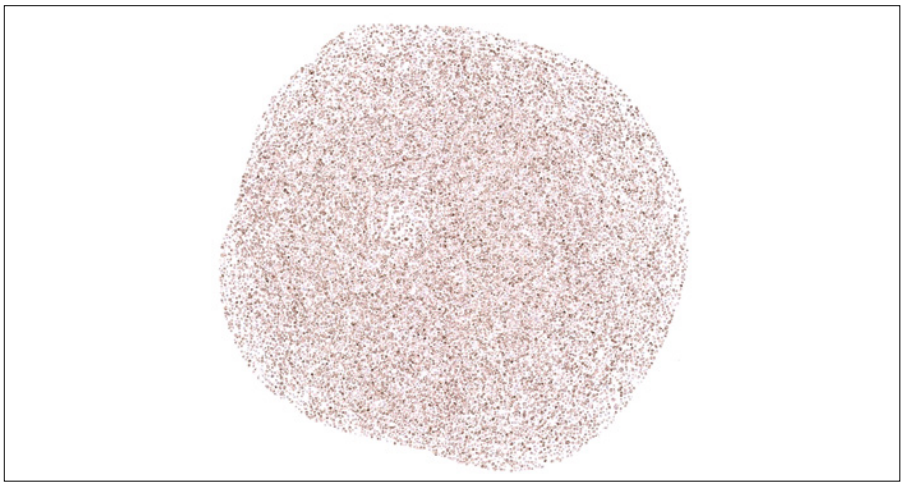


Figure 77: NCI-H226 cell pellet (2× magnification).

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

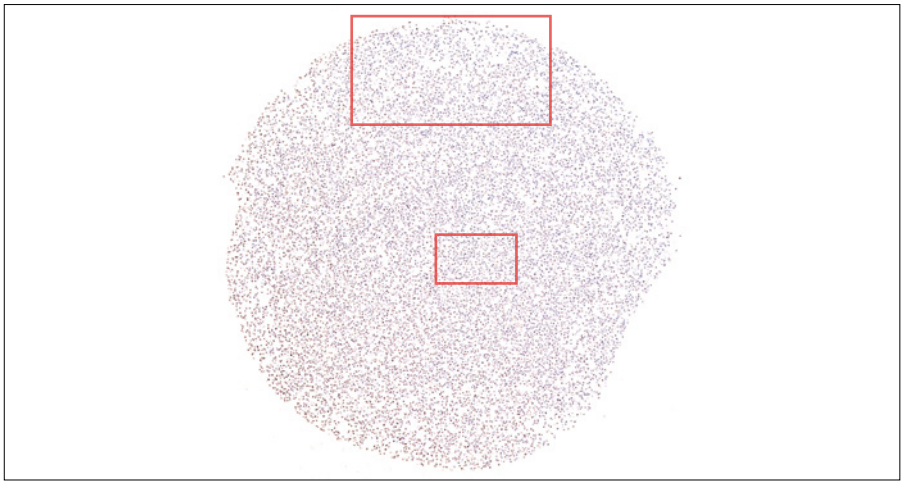


Figure 78: MCF-7 cell pellet (2× magnification).

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

Failed PD-L1 negative CCL (10×)

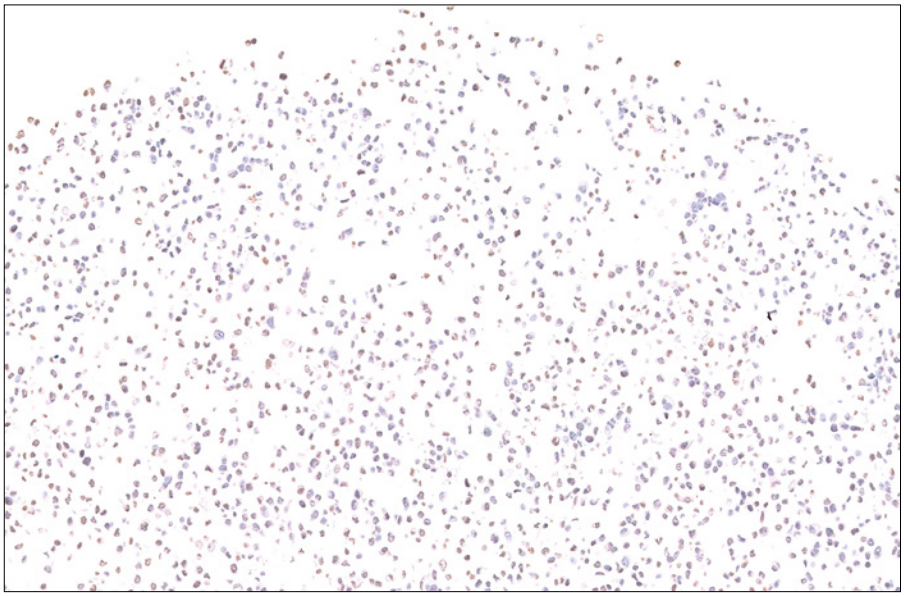


Figure 79: MCF-7 cell pellet (10× magnification).

Failed PD-L1 negative CCL (20×)

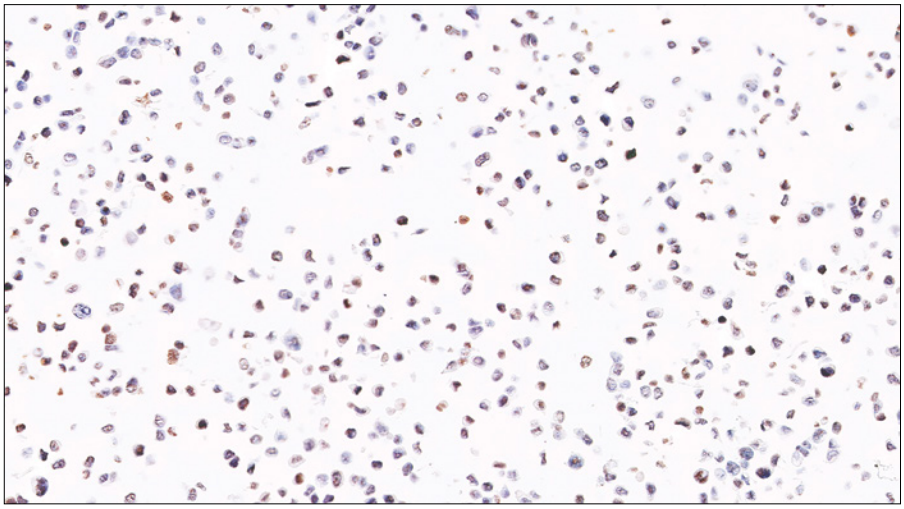


Figure 80: MCF-7 cell pellet (20× magnification).

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 protocol 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 1× EnVision FLEX Wash 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
	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	Use only EnVision FLEX Wash Buffer (20×) (Code K8007)
1× EnVision FLEX Target Retrieval Solution is cloudy in appearance when heated	When heated the 1× EnVision FLEX Target Retrieval Solution turns cloudy in appearance	This is normal and does not influence staining

Continued on next page

Problem	Probable Cause	Suggested Action
1× EnVision FLEX 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× EnVision FLEX Target Retrieval Solution. Do not modify the pH of 1× EnVision FLEX Target Retrieval Solution. If the pH is outside the acceptable range (6.1 ± 0.2), discard 1× EnVision FLEX Target Retrieval Solution. Prepare new 1× EnVision FLEX Target Retrieval Solution. Check the pH of the new 1× EnVision FLEX Target Retrieval Solution
	Inferior quality water is used to dilute the EnVision FLEX Target Retrieval Solution concentrate	Ensure that distilled or deionized water is used to prepare 1× EnVision FLEX Target Retrieval Solution
	Incorrect Target Retrieval Solution is used	Ensure that only EnVision FLEX Target Retrieval Solution, Low pH (50×) 1:50 (Code K8005) 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

KEYNOTE-826: Controlled Study of Combination Therapy in Patients with Persistent, Recurrent, or Metastatic Cervical Cancer

The efficacy of pembrolizumab in combination with paclitaxel and cisplatin or paclitaxel and carboplatin, with or without bevacizumab, was investigated in KEYNOTE 826, a multicenter, randomized, double-blind, placebo-controlled study that enrolled 617 patients with persistent, recurrent, or first-line metastatic cervical cancer who had not been treated with chemotherapy except when used concurrently as a radio-sensitizing agent. Patients were enrolled regardless of tumor PD-L1 expression status. Patients with autoimmune disease that required systemic therapy within 2 years of treatment or a medical condition that required immunosuppression were ineligible. Randomization was stratified by metastatic status at initial diagnosis, investigator decision to use bevacizumab, and PD-L1 status (CPS < 1 vs. CPS 1 to < 10 vs. CPS ≥ 10). Patients were randomized (1:1) to one of the two treatment groups:

- Treatment Group 1: Pembrolizumab 200 mg plus chemotherapy with or without bevacizumab
- Treatment Group 2: Placebo plus chemotherapy with or without bevacizumab

The investigator selected one of the following four treatment regimens prior to randomization:

1. Paclitaxel 175 mg/m^2 + cisplatin 50 mg/m^2
2. Paclitaxel 175 mg/m^2 + cisplatin 50 mg/m^2 + bevacizumab 15 mg/kg
3. Paclitaxel 175 mg/m^2 + carboplatin AUC 5 mg/mL/min
4. Paclitaxel 175 mg/m^2 + carboplatin AUC 5 mg/mL/min + bevacizumab 15 mg/kg

All study medications were administered as an intravenous infusion. All study treatments were administered on Day 1 of each 3 week treatment cycle. Cisplatin could be administered on Day 2 of each 3 week treatment cycle. The option to use bevacizumab was by investigator choice prior to randomization. Treatment with pembrolizumab continued until RECIST v1.1 defined progression of disease, unacceptable toxicity, or a maximum of 24 months. Administration of pembrolizumab was permitted beyond RECIST-defined disease progression if the patient was clinically stable and considered to be deriving clinical benefit by the investigator. Assessment of tumor status was performed at Week 9 and then every 9 weeks for the first year, followed by every 12 weeks thereafter.

Of the 617 enrolled patients, 548 patients (89%) had tumors expressing PD-L1 with a CPS ≥ 1 based on PD-L1 IHC 22C3 pharmDx. Among these 548 enrolled patients with tumors expressing PD-L1, 273 patients were randomized to pembrolizumab in combination with chemotherapy with or without bevacizumab, and 275 patients were randomized to placebo in combination with chemotherapy with or without bevacizumab. The baseline characteristics of these 548 patients were: median age of 51 years (range: 22 to 82), 16% age 65 or older; 59% White, 18% Asian, and 1% Black; 37% Hispanic or Latino; 56% and 43% ECOG performance status of 0 or 1, respectively; 63% received bevacizumab as study treatment; 21% with adenocarcinoma and 5% with adenosquamous histology; for patients with persistent or recurrent disease with or without distant metastases, 39% had received prior chemoradiation only and 17% had received prior chemoradiation plus surgery.

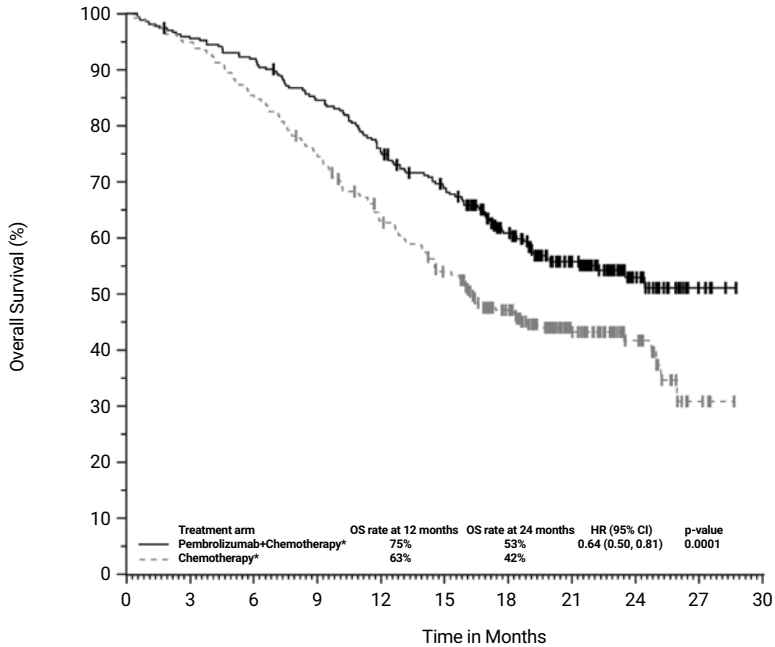
The primary efficacy outcome measures were OS and PFS as assessed by investigator according to RECIST v1.1. Secondary efficacy outcome measures were ORR and DoR, according to RECIST v1.1, as assessed by investigator.

The study demonstrated statistically significant improvements in OS and PFS for patients randomized to pembrolizumab in combination with chemotherapy with or without bevacizumab compared to placebo in combination with chemotherapy with or without bevacizumab at a pre-specified interim analysis in the overall population. The median follow-up time was 17.2 months (range: 0.3 to 29.4 months). Table 5 summarizes key efficacy measures for patients whose tumors expressed PD-L1 with a CPS ≥ 1 in KEYNOTE-826 from the pre-specified interim analysis. The Kaplan-Meier curves for OS and PFS are shown in Figures 81 and 82.

Table 5: Efficacy Results in KEYNOTE-826 for Patients with PD-L1 Expression (CPS ≥ 1)

Endpoint	Pembrolizumab 200 mg every 3 weeks plus Chemotherapy* with or without bevacizumab n=273	Placebo plus Chemotherapy* with or without bevacizumab n=275
OS		
Number of patients with event (%)	118 (43)	154 (56)
Median in months (95% CI)	NR (19.8, NR)	16.3 (14.5, 19.4)
Hazard ratio† (95% CI)	0.64 (0.50, 0.81)	
p-Value‡	0.0001	
PFS		
Number of patients with event (%)	157 (58)	198 (72)
Median in months (95% CI)	10.4 (9.7, 12.3)	8.2 (6.3, 8.5)
Hazard ratio† (95% CI)	0.62 (0.50, 0.77)	
p-Value§	< 0.0001	
Objective Response Rate		
ORR¶ (95% CI)	68% (62, 74)	50% (44, 56)
Complete response rate	23%	13%
Partial response rate	45%	37%
Duration of Response		
Median in months (range)	18.0 (1.3+, 24.2+)	10.4 (1.5+, 22.0+)
% of patients with duration ≥ 12 months#	56	46

** Chemotherapy (paclitaxel and cisplatin or paclitaxel and carboplatin)*
† Based on the stratified Cox proportional hazard model
‡ Based on stratified log-rank test (compared to an alpha boundary of 0.00549)
§ Based on stratified log-rank test (compared to an alpha boundary of 0.00144)
¶ Response: Best objective response as confirmed complete response or partial response
 # Based on Kaplan-Meier estimation
NR = not reached

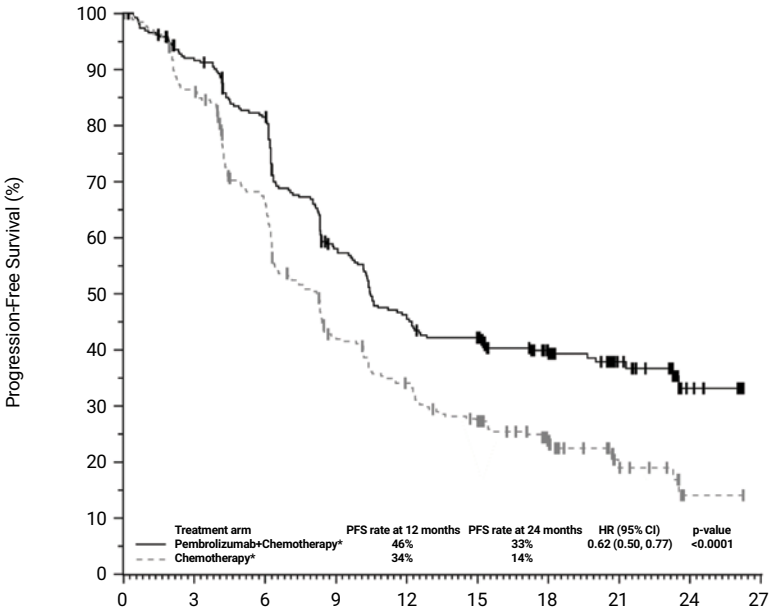


Number at Risk

Pembrolizumab + Chemotherapy*:	273	260	250	229	204	181	132	82	34	6	0
Chemotherapy*:	275	261	235	206	168	140	100	55	25	4	0

Figure 81: Kaplan-Meier Curve for Overall Survival by Treatment Arm in KEYNOTE-826 Patients with PD-L1 Expression (CPS ≥ 1).

** Chemotherapy (paclitaxel and cisplatin or paclitaxel and carboplatin) with or without bevacizumab*



Number at Risk

Pembrolizumab + Chemotherapy*:	273	238	208	143	112	101	66	34	10	0
Chemotherapy*:	275	229	170	103	81	63	38	13	1	0

Figure 82: Kaplan-Meier Curve for Progression Free Survival by Treatment Arm in KEYNOTE-826 Patients with PD-L1 Expression (CPS ≥ 1).

** Chemotherapy (paclitaxel and cisplatin or paclitaxel and carboplatin) with or without bevacizumab*

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a. PD-L1 IHC 22C3 pharmDx [Instructions for Use]. Santa Clara, CA: Agilent Technologies, Inc.; 2022. **b.** Keytruda [Summary of Product Characteristics]. European Medicines Agency; 2022. **c.** Data on file. Agilent Technologies, Inc.

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D70829_01

Published in the USA, May 16, 2022
29493 2022APR25

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