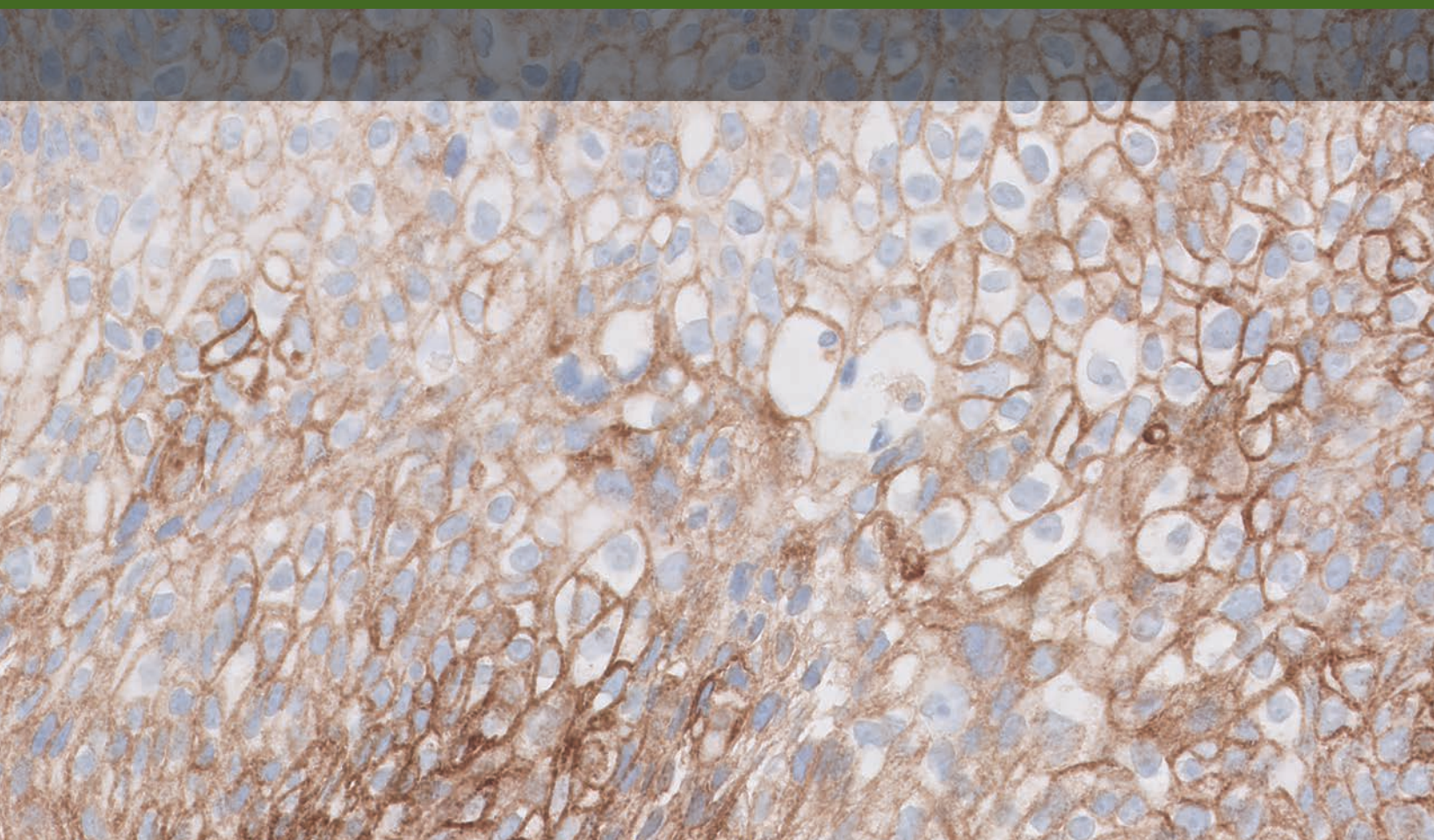


PD-L1 IHC 22C3 pharmDx Interpretation Manual – Head and Neck Squamous Cell Carcinoma (HNSCC)

For in vitro diagnostic use

Rx only



This Interpretation Manual can be used for:

- PD-L1 IHC 22C3 pharmDx, Code SK006, for use with Autostainer Link 48 and PT Link
- PD-L1 IHC 22C3 pharmDx, Code GE006, for use with Dako Omnis

PD-L1 IHC 22C3 pharmDx refers to both Code SK006 and Code GE006.

PD-L1 IHC 22C3 pharmDx, Code SK006 or Code GE006, is not available in all countries.

Refer to the pharmDx Code SK006 and Code GE006 product pages on agilent.com to confirm product availability in your country.

Refer to the country-specific PD-L1 IHC 22C3 pharmDx, Code SK006 or Code GE006 Instructions for Use (IFU) for the detailed intended use statement specifying indications and expression level cutoff values.

All images were stained with PD-L1 IHC 22C3 pharmDx, Code SK006 unless otherwise indicated.

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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) head and neck squamous cell carcinoma (HNSCC) tissue using EnVision FLEX visualization system on Autostainer Link 48 (Code SK006) and Dako Omnis (Code GE006).

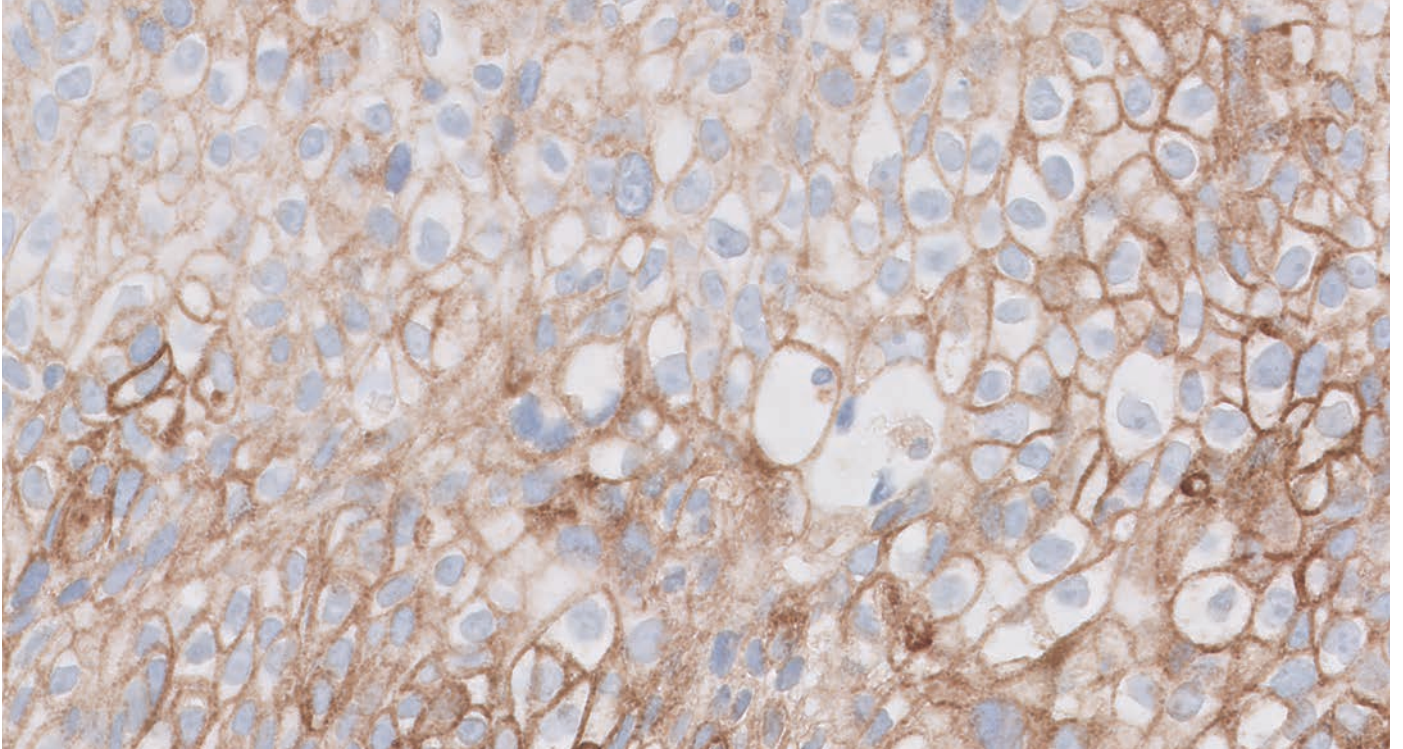
Head and Neck Squamous Cell Carcinoma (HNSCC)

PD-L1 protein expression in HNSCC 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. The specimen should be considered to have PD-L1 expression if $CPS \geq 1$.

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

Refer to the country-specific PD-L1 IHC 22C3 pharmDx, Code SK006 or Code GE006 Instructions for Use (IFU) for the detailed intended use statement specifying indications and expression level cutoff values.

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



Introduction

PD-L1 IHC 22C3 pharmDx is the only clinical trial-proven companion diagnostic indicated as an aid in identifying patients with head and neck squamous cell carcinoma (HNSCC) for treatment with KEYTRUDA® (pembrolizumab). This Interpretation Manual is provided as a tool to help guide pathologists and laboratory personnel in achieving correct and reproducible results in assessing PD-L1 expression in FFPE HNSCC specimens.

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

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

HNSCC 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
- CPS ≥ 20

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

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 25.

Photomicrographs

The included photomicrographs are of HNSCC unless otherwise noted.

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

Tissue samples were provided by the Cooperative Human Tissue Network which is funded by the National Cancer Institute. Other investigators may have received specimens from the same subjects.

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

Technical Considerations

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

Specimen Preparation

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

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
 - Kit-supplied and required as a run control to verify reagent performance with Code SK006
 - Not included and not a required run control with Code GE006, but available to purchase as product code T1391 for optional use
 - Refer to page 11 and the Control Cell Line (CCL) Appendix of this interpretation manual on page 60 for CCL pellet expected staining, acceptance criteria, and example images to guide interpretation
- Positive and negative lab-supplied control tissues stained with the primary antibody and Negative Control Reagent*
- Serial section of each patient specimen stained with the Negative Control Reagent*

** For Code GE006 testing, refer to your country-specific IFU for requirements and recommendations regarding the use Negative Control Reagent-stained positive and negative lab-supplied control tissue slides and patient slide*

Lab-supplied Control Tissue

Include positive and negative lab-supplied control tissue slides within each staining run.

Refer to the 'Recommended Order of Slide Evaluation' table in the PD-L1 IHC 22C3 pharmDx IFU, Code SK006 or Code GE006 for your country, for additional information on appropriate control tissue. Fix, process, and embed the control tissue in the same manner as the patient specimen. Control tissues processed differently from the patient specimen validate reagent performance only and do not verify tissue preparation.

The ideal positive control tissue (slide stained with PD-L1) provides a complete dynamic representation of weak-to-moderate staining of tumor cells and may contain PD-L1 expressing tumor-associated immune cells.

The ideal negative control tissue (slide stained with PD-L1) demonstrates no membrane staining on tumor cells. The tissue may contain tumor-associated immune cells that express PD-L1 and offer an internal positive control, but this should be verified by the user. Alternatively, negative portions of the positive control tissue may serve as the negative control tissue, but this should be verified by the user.

Optional Additional Lab-supplied Control: Tonsil Tissue

Tonsil tissue stained with PD-L1 should be prescreened to exhibit strong staining in portions of the crypt epithelium and weak-to-moderate staining of the follicular macrophages in the germinal centers. PD-L1 expression of the endothelium, fibroblasts, as well as the surface epithelium should be negative. Tonsil control tissue is optional for use with Code SK006 and has not been included for use with Code GE006.

Tissue Processing

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

Cut tissue specimens into sections of 4–5 µm. After sectioning, tissues should be mounted on Dako FLEX IHC Microscope Slides (Code K8020) or Superfrost Plus slides, and then placed in a 58 ± 2 °C oven for 1 hour. To preserve antigenicity, store tissue sections in the dark at 2–8 °C (preferred) or at room temperature up to 25 °C in the dark and stain within the time period specified in the country-specific Code SK006 or Code GE006 IFU for each indication and temperature condition.

For details on assay storage and staining procedures, refer to the country-specific PD-L1 IHC 22C3 pharmDx, Code SK006 or Code GE006 IFU.

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 24. 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, IHC staining of the remaining 2 serial sections is likely to be acceptable.

Each PD-L1 IHC 22C3 pharmDx, Code SK006 kit is configured with Control Cell Line Slides that should be included in each Code SK006 IHC run. Guidelines on interpreting the Control Cell Line Slide are reviewed on the next page.

Lab-supplied control tissue slides should also be assessed with every IHC run.

Specimen Adequacy

Confirm the Presence of at Least 100 Viable Tumor Cells

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

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

Instructions for Patient Specimens With Less Than 100 Viable Tumor Cells

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

Evaluating Controls

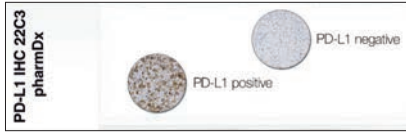


Figure 1: Each Control Cell Line Slide contains sections of cell pellets with positive and negative PD-L1 expression.

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 1). Assess the percentage of positive cells, staining intensity, and nonspecific staining in both cell pellets. If any staining of the Control Cell Line Slide is not satisfactory, all results with the patient specimens should be considered invalid. Do not use the Control Cell Line Slide as an aid in interpretation of patient results.

Evaluate the overall staining intensity using the following guide:

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

Positive Control Cell Pellet

The following staining is acceptable for the PD-L1 positive cell pellet (Figure 2):

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

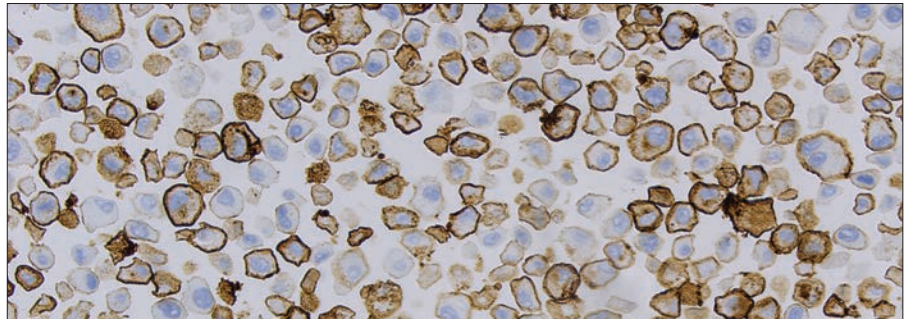


Figure 2: Positive cell pellet with acceptable staining of PD-L1 IHC 22C3 pharmDx Control Cell Line Slide (20 \times magnification).

Negative Control Cell Pellet

For the PD-L1 negative cell pellet, the following staining is acceptable (Figure 3):

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

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

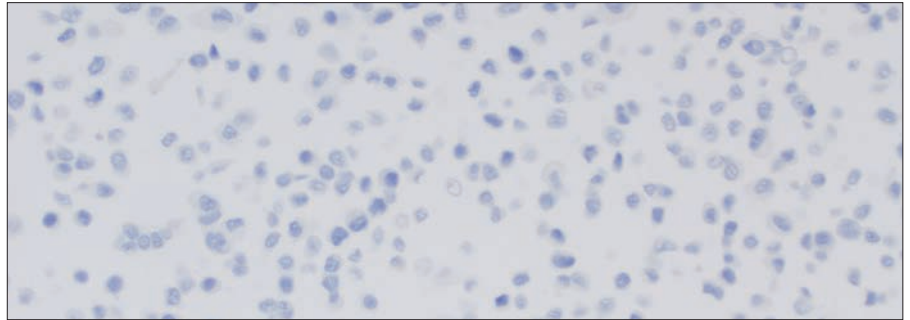


Figure 3: Negative cell pellet with no staining of PD-L1 IHC 22C3 pharmDx Control Cell Line Slide (20 \times magnification).

See the Control Cell Line (CCL) Appendix on page 60 for images of passing, borderline and failing control cell line staining.

Positive Lab-supplied Control Tissue

Examine the positive lab-supplied control tissue slides to determine that the tissues are correctly prepared and reagents are functioning properly. The ideal positive control tissue (slide stained with PD-L1) provides a complete dynamic representation of weak-to-moderate staining of tumor cells and may contain PD-L1 expressing tumor-associated immune cells (Figure 4). If staining of positive lab-supplied control tissue slides is not satisfactory, all results with the patient specimen should be considered invalid.

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

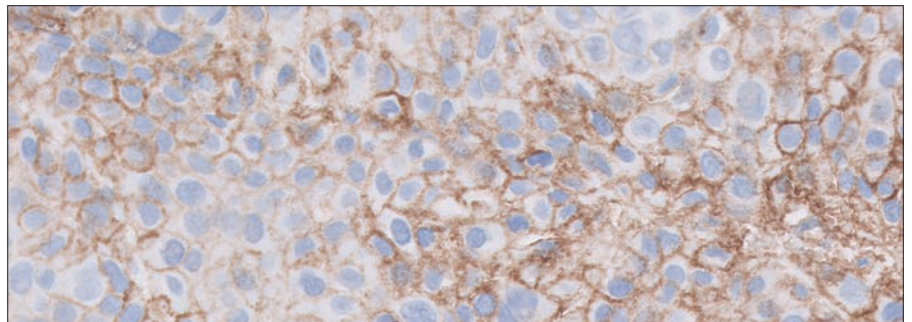


Figure 4: Ideal positive lab-supplied control tissue slide stained with PD-L1 primary antibody (20× magnification).

Negative Lab-supplied Control Tissue

Examine the Negative Control Tissue slides to verify the specificity of the labeling of the target antigen by the primary antibody. The ideal negative control tissue (slide stained with PD-L1) demonstrates no membrane staining on tumor cells. The tissue may contain tumor-associated immune cells that express PD-L1 and offer an internal positive control, but this should be verified by the user (Figure 5). Alternatively, negative portions of the positive control tissue may serve as the negative control tissue, but this should be verified by the user. Examine the negative lab-supplied control tissue slides to determine the expected staining.

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

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

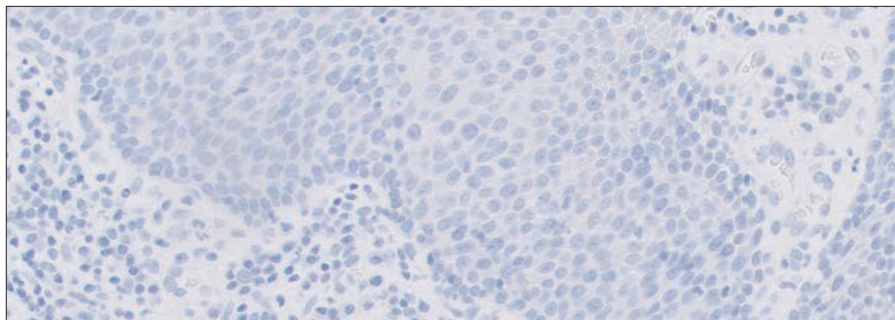


Figure 5: Ideal negative lab-supplied control tissue slide stained with PD-L1 primary antibody demonstrating lack of membrane staining on tumor cells (20 \times magnification).

Optional Additional Control Tissue

In addition to the Control Cell Line Slide and lab-supplied control tissues, FFPE tonsil tissue may also be used as an optional control specimen. Tonsil tissue 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 6).

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

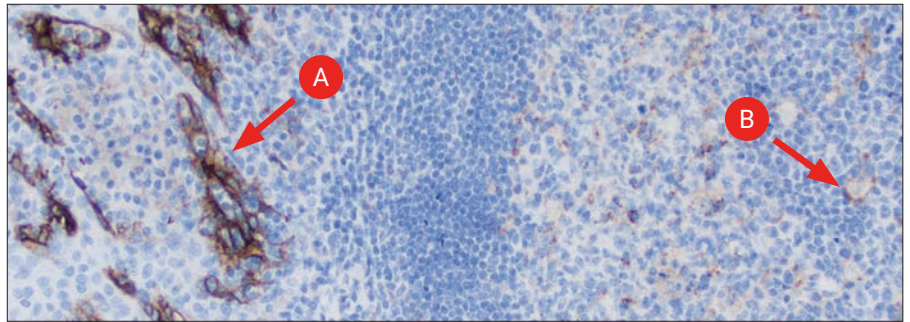


Figure 6: Tonsil tissue stained with PD-L1 primary antibody exhibiting strong membrane staining in portions of the crypt epithelium (A) and weak-to-moderate membrane staining of follicular macrophages in the germinal centers (B) (10× magnification).

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

Negative Control Reagent (NCR)

Examine the patient slides stained with the NCR to identify nonspecific staining, including nuclear staining that may interfere with PD-L1 staining interpretation, making the specimen non-evaluable. Satisfactory performance is indicated by the absence of cell membrane staining (Figure 7).

Examine the patient slide stained with the NCR to determine if there is any nonspecific staining that may interfere with interpreting the PD-L1 stained slide.

Nonspecific staining, including nuclear staining, should be $\leq 1+$ in scorable tumor regions.

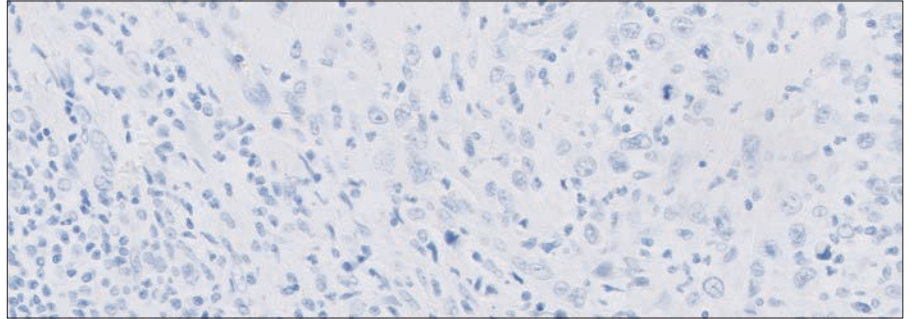


Figure 7: HNSCC tissue specimen slide stained with NCR (20× magnification).

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

Slide Evaluation Flowchart



* Optional use with PD-L1 IHC 22C3 pharmDx, Code GE006

Figure 8: Recommended order of slide evaluation.

Combined Positive Score

Definition of Combined Positive Score (CPS)

PD-L1 expression in HNSCC 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:

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

** Macrophages and histiocytes are considered the same cells*

CPS Numerator Inclusion and Exclusion Criteria

Any perceptible and convincing partial or complete linear membrane staining ($\geq 1+$) 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 ($\geq 1+$) 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 20 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 nonstaining 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 (biopsies or resections) for the specimen to be considered adequate for evaluation. Note: Use of PD-L1 IHC 22C3 pharmDx on fine needle aspirates has not been validated
- 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 nonstaining (CPS denominator)
 - Determine the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) (CPS numerator; see Tables 1 and 2 on page 18 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

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	<ul style="list-style-type: none"> – Nonstaining tumor cells – Tumor cells with only cytoplasmic staining – 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 [†] : <ul style="list-style-type: none"> – Lymphocytes (including lymphocyte aggregates) – Macrophages[‡] Only MICs directly associated with the response to the tumor are scored	<ul style="list-style-type: none"> – Nonstaining MICs – MICs (including lymphoid aggregates) associated with ulcers or other inflammatory processes – MICs associated with carcinoma in situ – MICs associated with benign structures – Neutrophils, eosinophils, and plasma cells
Other Cells	Not included	<ul style="list-style-type: none"> – Benign cells – 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 are included in the score

[†] Adjacent MICs are defined as being within the same 20x 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

Tissue Elements	Included in the Denominator	Excluded from the Denominator
Tumor Cells	All viable invasive tumor cells	<ul style="list-style-type: none"> – Any necrotic or nonviable tumor cells – Carcinoma in situ (CIS)
Immune Cells	Not included	All immune cells of any type
Other Cells	Not included	<ul style="list-style-type: none"> – Benign cells – 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.

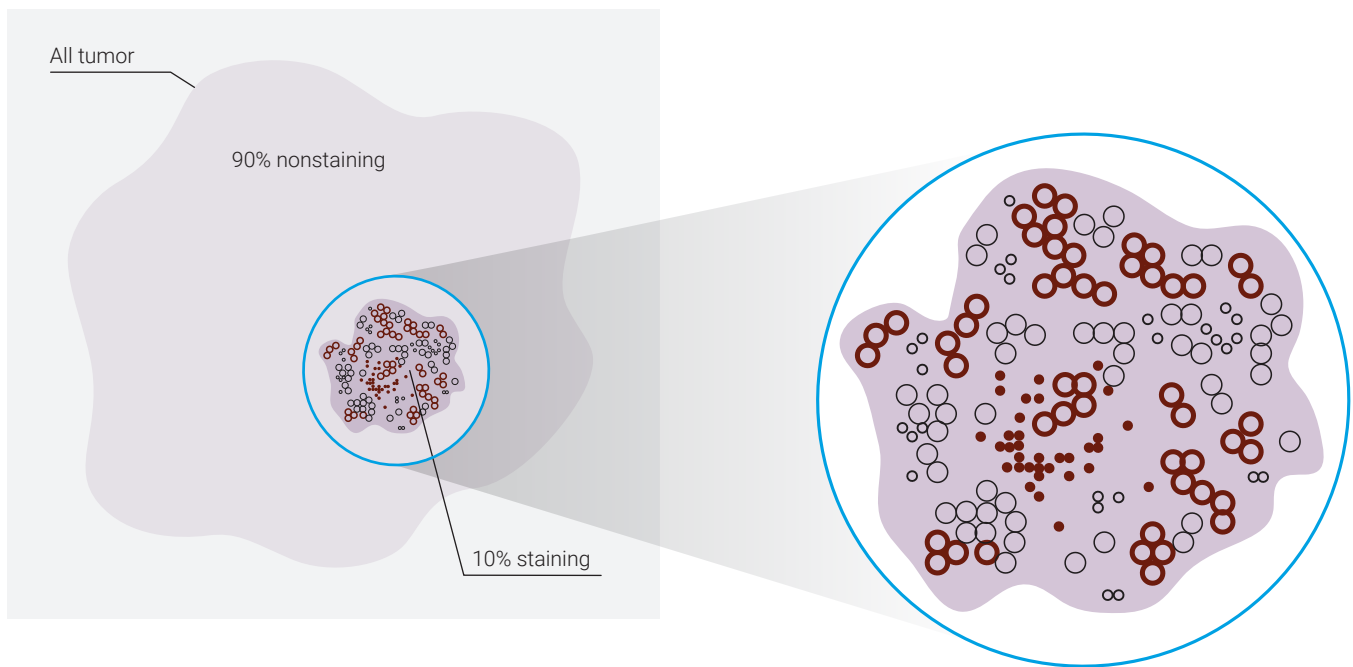
Example 1: Calculation of Combined Positive Score Based on a Small PD-L1 Staining Area

First: Evaluate the tumor area for perceptible and convincing staining as described in “Determining Combined Positive Score” on page 19.

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:

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

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

- PD-L1 staining tumor cell
- Nonstaining tumor cell
- PD-L1 staining mononuclear inflammatory cell
- Nonstaining mononuclear inflammatory cell

Clinical Interpretation: CPS ≥ 1

[§] Including tumor cells, lymphocytes, macrophages

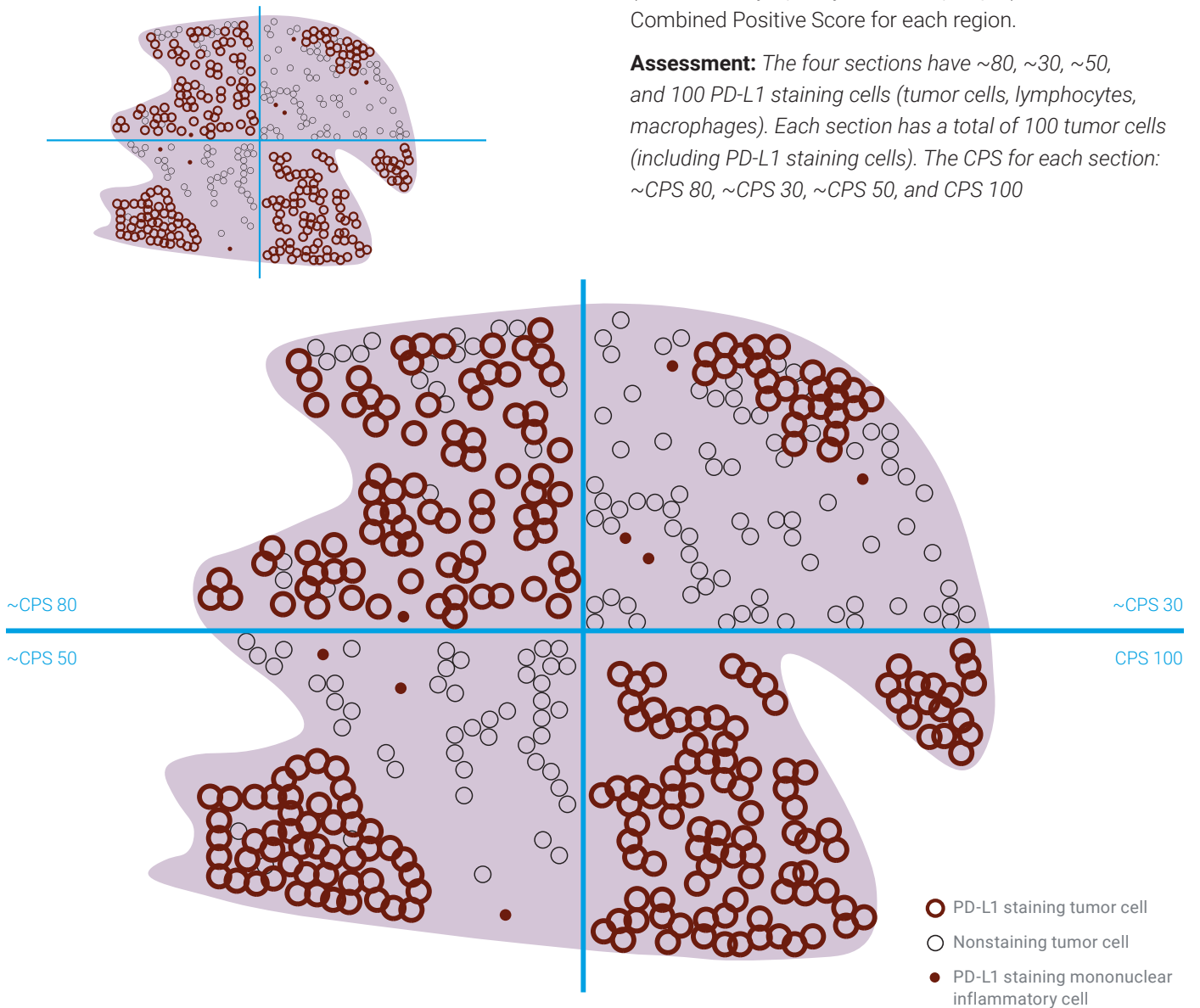
Figure 9: Example of tumor with small PD-L1 staining area.

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:

$$(80+30+50+100 / 4) \times 100 \approx \text{CPS } 65$$

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

Clinical Interpretation: CPS ≥ 20

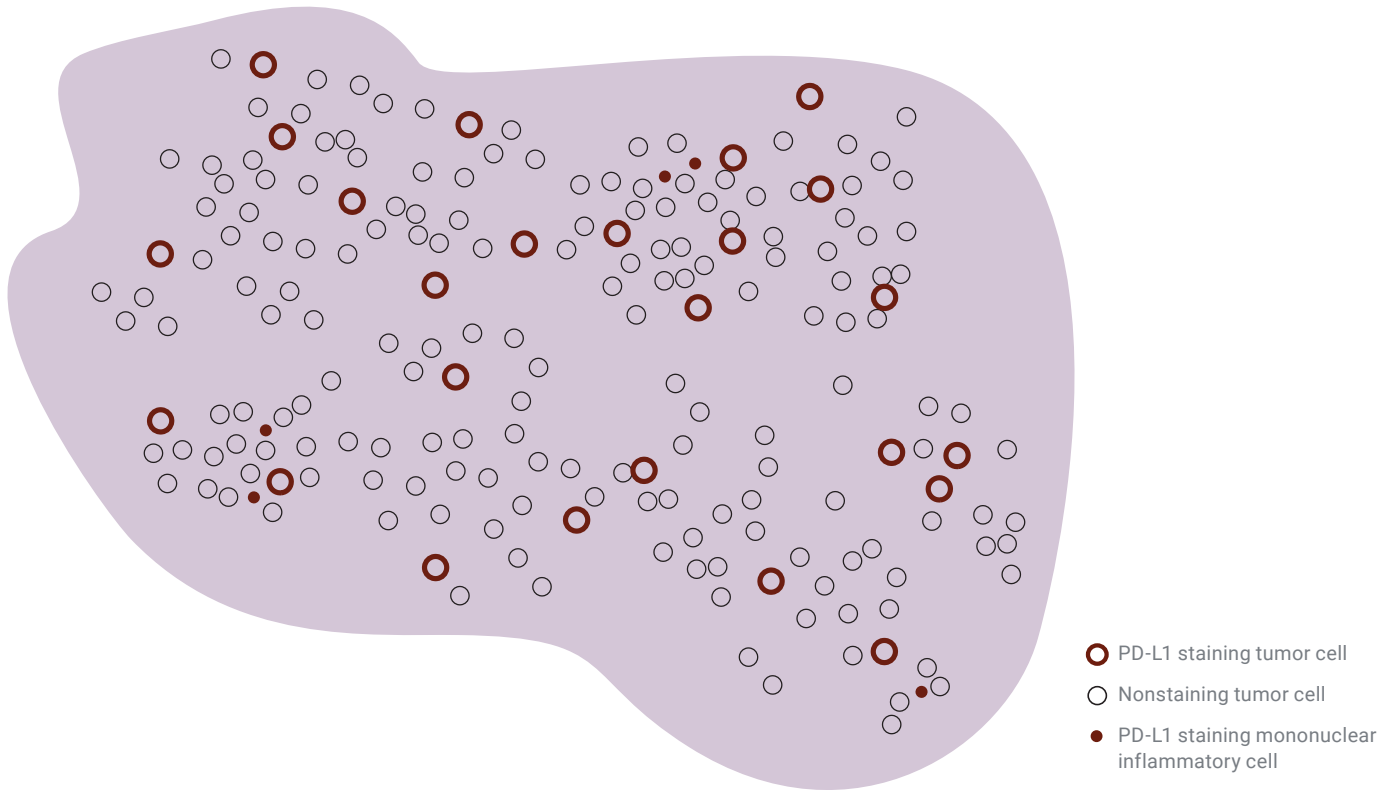
Figure 10: Example with heterogeneous PD-L1 staining area.

Example 3: Calculation of Combined Positive Score for a Homogeneous Staining Specimen

First: Evaluate the specimen for perceptible and convincing staining as described in “Determining Combined Positive Score” on page 19.

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 nonstaining areas) and estimate the total number of viable tumor cells (PD-L1 staining and nonstaining tumor cells). Calculate the Combined Positive Score.

Assessment: Tumor specimen has perceptible and convincing staining. There are 30 PD-L1 staining cells (tumor cells, lymphocytes, macrophages). 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:

$$CPS = \frac{\# \text{ PD-L1 staining cells}^*}{\text{Total \# viable tumor cells}} \times 100 = \frac{30 \text{ PD-L1 staining cells}}{200 \text{ tumor cells}} \times 100 = CPS 15$$

Clinical Interpretation: CPS ≥ 1

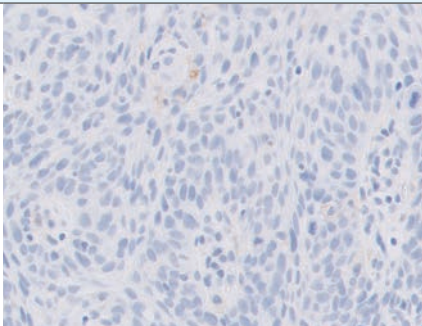
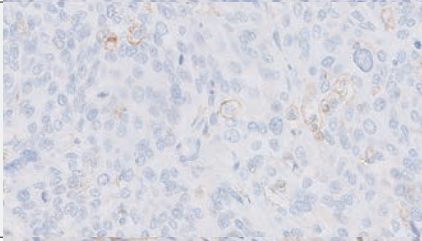
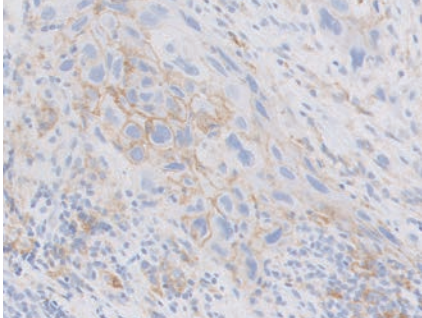
* Including tumor cells, lymphocytes, macrophages

Figure 11: Example of homogeneous staining specimen.

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	
≥ 20	CPS is greater than or equal to 20	

Note: PD-L1 expression level CPS ≥ 20 may be of interest to treating physician but does not determine eligibility for first-line treatment with KEYTRUDA® (pembrolizumab) as a single agent.

Clinicians should use caution when interpreting test results at the CPS ≥ 20 cutoff because PD-L1 IHC 22C3 pharmDx, Code SK006 failed to meet prespecified acceptance criteria for positive percent agreement in two independent inter-site reproducibility studies and overall percent agreement in one inter-site reproducibility study conducted on HNSCC specimens at the CPS ≥ 20 cutoff. All prespecified acceptance criteria were met for HNSCC specimens at the CPS ≥ 1 cutoff, including the independent inter-site reproducibility.

Due to the nature of IHC, it is recommended that challenging borderline cases with scores near the cutoff are reviewed by a second pathologist and consensus results be provided for these cases.

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 Code SK006 or Code GE006: _____

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 \geq 1:

CPS $\geq 20^{\circ}$:

Note: Due to the nature of IHC, it is recommended that challenging borderline cases with scores near the cutoff are reviewed by a second pathologist and consensus results be provided for these cases.

* Optional use with PD-L1 IHC 22C3 pharmDx, Code GE006

$^{\circ}$ PD-L1 expression level CPS ≥ 20 may be of interest to treating physician but does not determine eligibility for first-line treatment with KEYTRUDA[®] (pembrolizumab) as a single agent

Combined Positive Score Summary and Examples

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

By definition, PD-L1 staining cells in HNSCC 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 level in HNSCC 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.

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

This section will define and illustrate scoring inclusions and exclusions for accurate determination of Combined Positive Score. All images are HNSCC unless otherwise noted.

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

PD-L1 Staining 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 page 20 for additional CPS inclusion/exclusion criteria). All viable tumor cells should be included in the denominator. Below are common staining characteristics of PD-L1 staining cells that must be included in the CPS numerator. All images are HNSCC unless otherwise noted in the figure caption.

Tumor Cells

Linear Membrane Staining

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

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

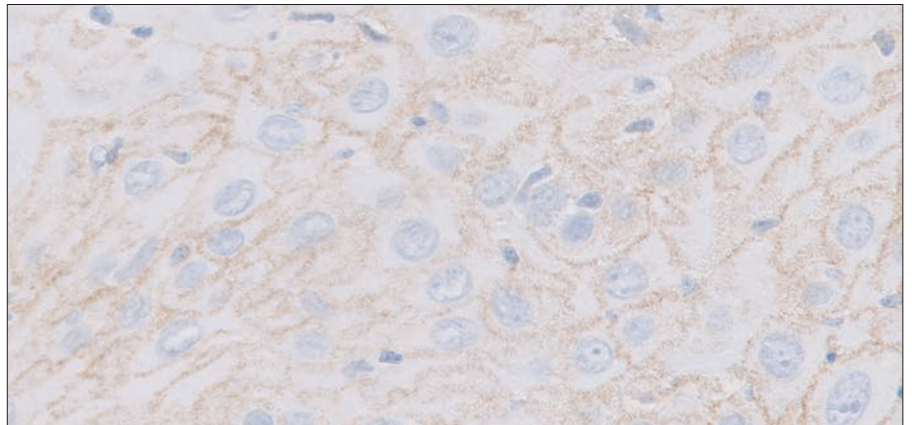


Figure 12a: HNSCC specimen stained with PD-L1 primary antibody exhibiting 1+ linear membrane staining of tumor cells (20× magnification).

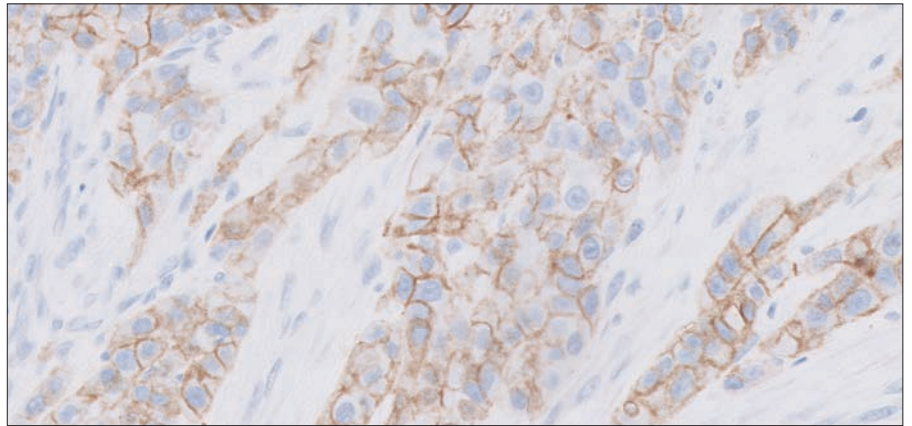


Figure 12b: HNSCC specimen stained with PD-L1 primary antibody exhibiting 2+ linear membrane staining of tumor cells (20× magnification).

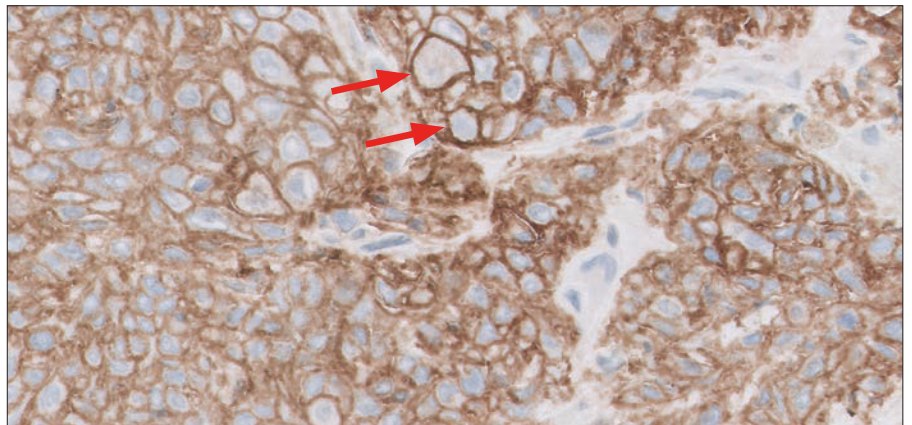


Figure 12c: HNSCC specimen stained with PD-L1 primary antibody exhibiting 3+ linear membrane staining of tumor cells (arrows) (20× magnification).

Key Point

Perceptible and convincing linear membrane staining of tumor cells at any intensity should be included in the CPS numerator

Partial Linear Membrane Staining

Tumor cells can exhibit partial linear membrane staining. Any partial linear membrane staining observed at any intensity at a 20× magnification must be included in the CPS numerator.

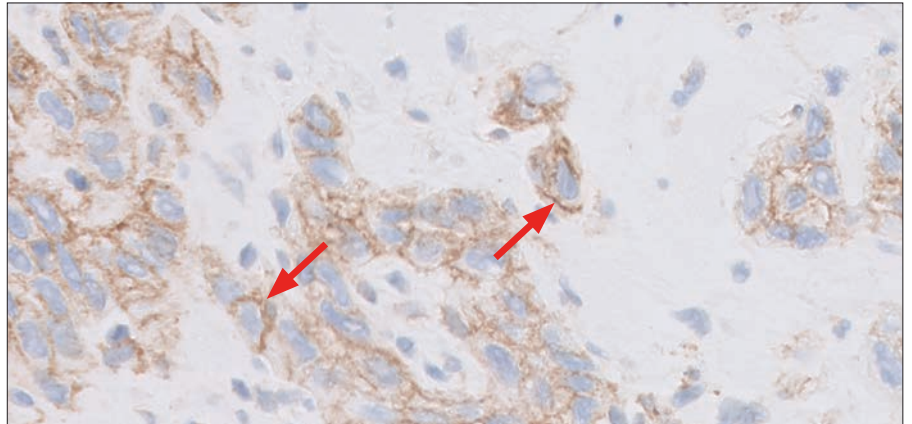


Figure 13: HNSCC specimen stained with PD-L1 primary antibody exhibiting partial linear membrane staining of tumor cells (arrows) (20× magnification).

Key Point

Perceptible and convincing partial linear membrane staining of tumor cells should be included in the CPS numerator

Linear Membrane and Cytoplasmic Staining

Tumor cells with both perceptible and convincing linear membrane staining ($\geq 1+$ 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. Additionally, linear PD-L1 staining of tumor cells can be smooth or granular. If partial or complete linear membrane staining is distinct from cytoplasmic staining, then the cell should be included in the CPS numerator.

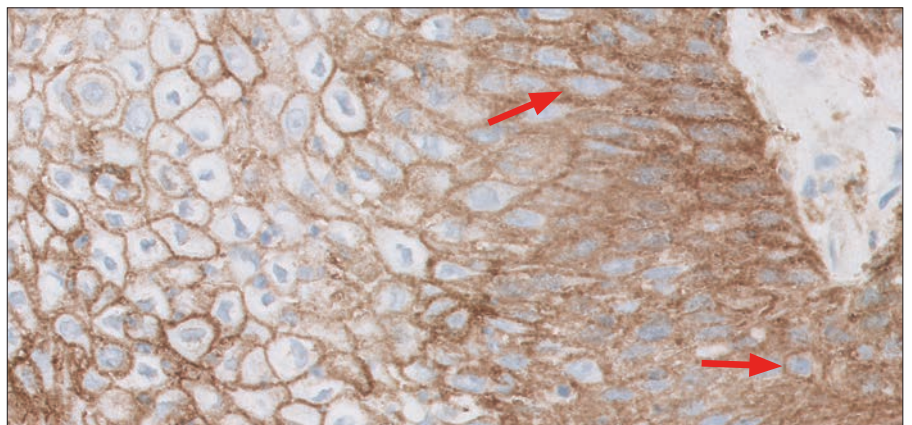


Figure 14: HNSCC specimen stained with PD-L1 primary antibody exhibiting linear membrane staining distinct from cytoplasmic staining (arrows) (20× magnification).

Key Point

Tumor cells exhibiting perceptible and convincing linear membrane staining that is distinct from cytoplasmic staining are included in the CPS numerator

Granular Staining

Tumor cells can exhibit a granular membrane staining pattern where membrane and cytoplasmic staining are indistinguishable. Only perceptible and convincing membrane staining of tumor cells ($\geq 1+$ intensity) observed at no higher than 20 \times magnification should be included in the CPS numerator.

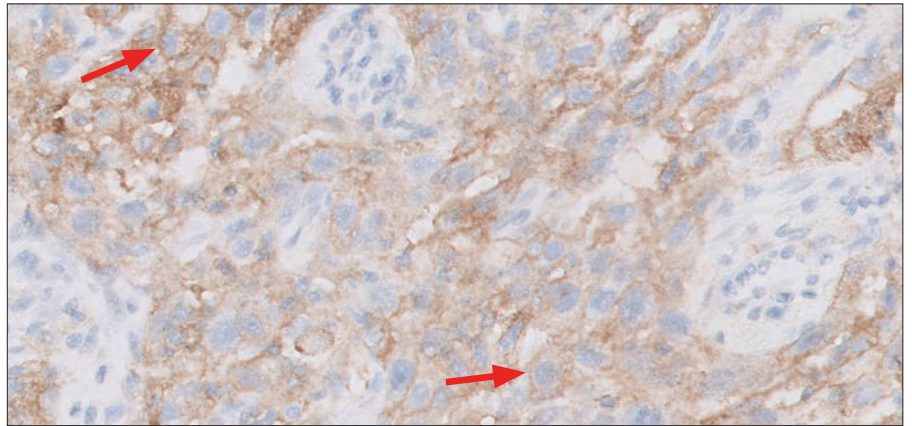


Figure 15: HNSCC specimen stained with PD-L1 primary antibody exhibiting granular linear membrane staining pattern (arrows) (20 \times magnification).

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 HNSCC 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. Additionally, multinucleate macrophages are commonly seen in HNSCC and, if PD-L1 staining is present on these cells, they should be counted as one cell and included in the numerator.

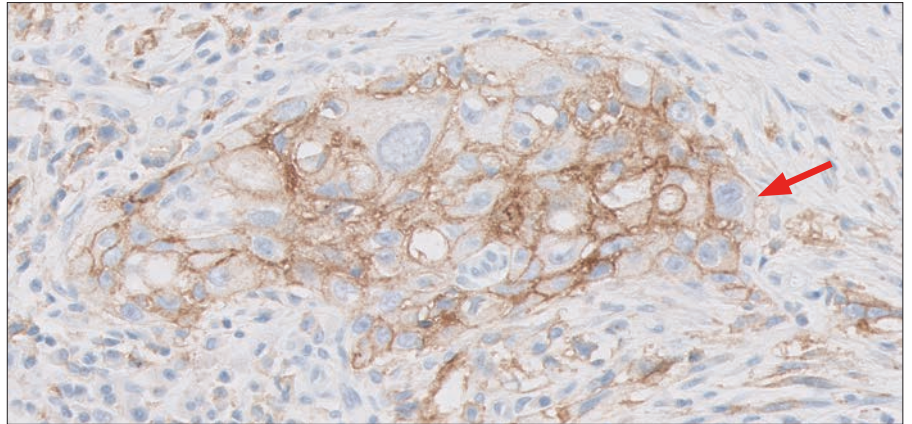


Figure 16: Multinucleate tumor cell (arrow) (20× magnification).

Key Point

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

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 ($\geq 1+$ 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.

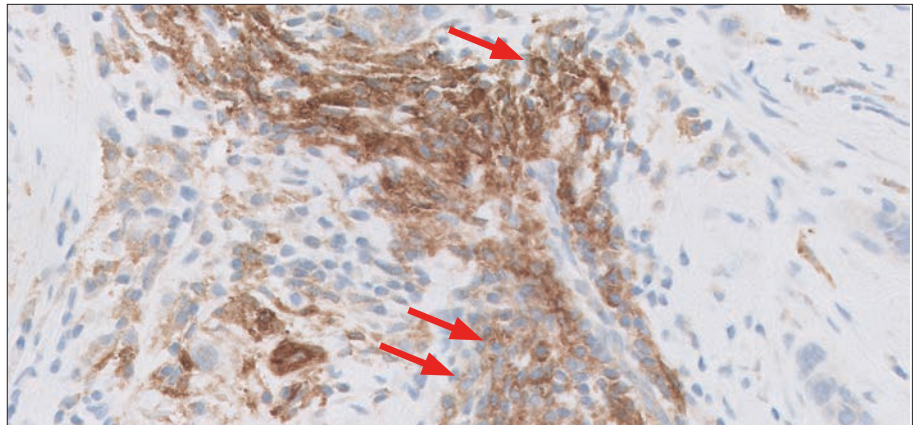


Figure 17a: HNSCC specimen stained with PD-L1 primary antibody exhibiting staining of tumor-associated lymphocytes (arrows) (20× magnification).

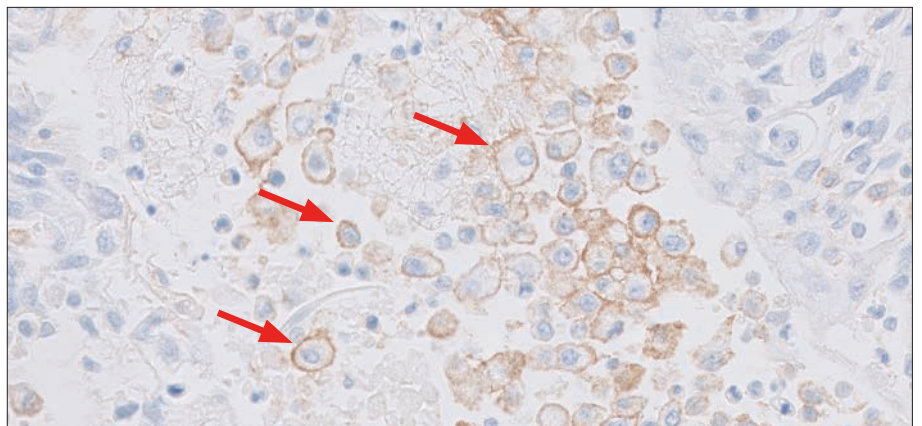


Figure 17b: HNSCC specimen stained with PD-L1 primary antibody exhibiting staining of tumor-associated macrophages (arrows) (20× magnification).

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. In general, include PD-L1 staining MICs that are within 0.5 mm of the tumor cells. This rule may be applied to tumors within lymph nodes that contain PD-L1 staining MICs. See Figures 18a–18c for an example of determining which MICs are included in the CPS numerator.

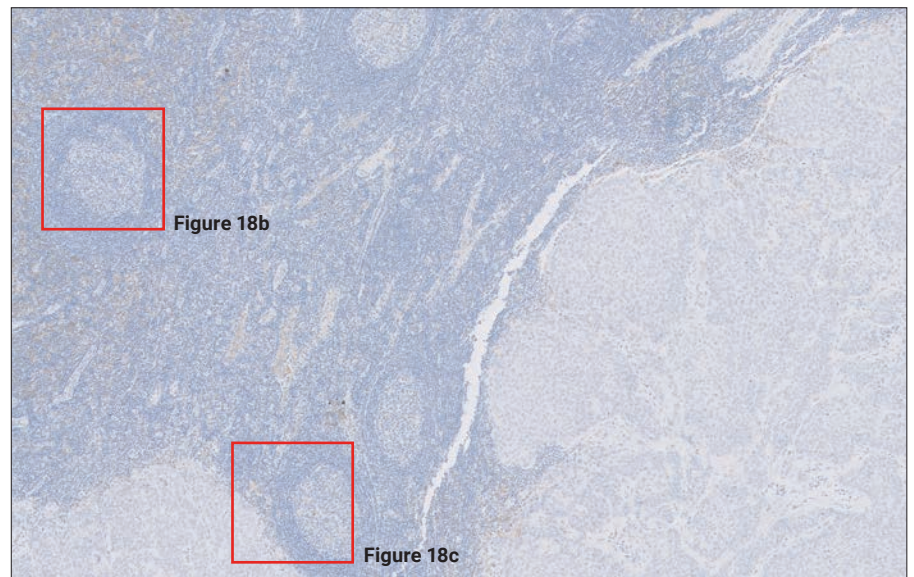


Figure 18a: At 5× magnification, several areas of PD-L1 staining mononuclear inflammatory cells are visible. To demonstrate which immune cells to include in the numerator, zoom in to 20× magnification on the boxed fields (5× magnification).

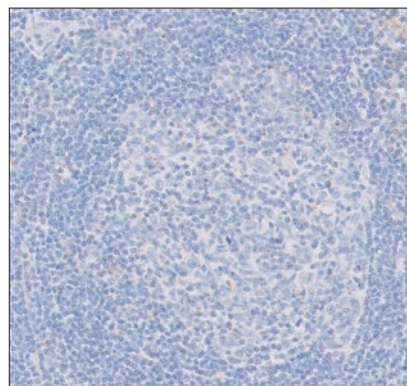


Figure 18b: Tumor cells are absent from this 20× field containing PD-L1 staining mononuclear inflammatory cells, thus none of these cells should be included in the numerator (20× magnification).

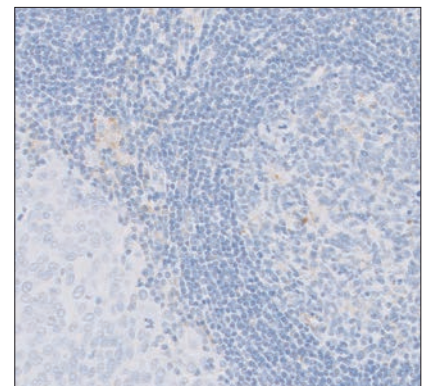


Figure 18c: When positioning the tumor cells in the approximate center of a 20× field, PD-L1 staining mononuclear inflammatory cells that are present within the same field should be included in the numerator (20× magnification).

Tumor Cell Density Patterns

HNSCC includes different morphologies that can impact the Combined Positive Score (CPS) by increasing or decreasing the total number of tumor cells that are included in the denominator. A squamous cell pattern with well-differentiated, cytoplasmic rich tumor cells will commonly have fewer cells per 20× field, whereas a poorly-differentiated, basaloid pattern will commonly have a higher number of tumor cells per 20× field. 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 fill a 20× field, there would be approximately 2500 tumor cells in that field.

Moderate Density Pattern

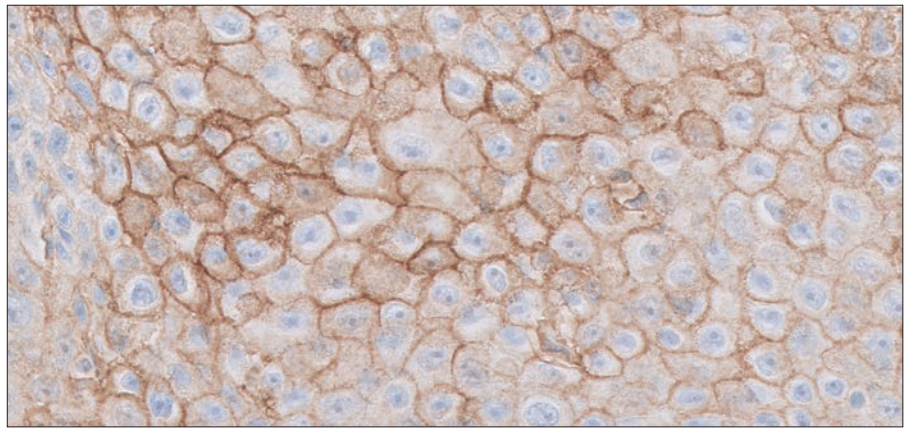


Figure 19: HNSCC specimen stained with PD-L1 primary antibody exhibiting moderately differentiated tumor cell pattern (20× magnification).

High Density Pattern

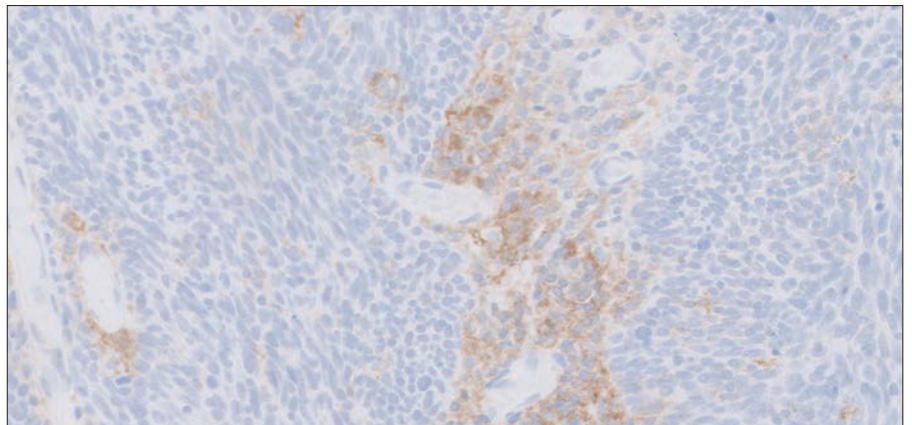


Figure 20: HNSCC specimen stained with PD-L1 primary antibody exhibiting poorly differentiated, basaloid tumor cell pattern (20× magnification).

Low Density Pattern

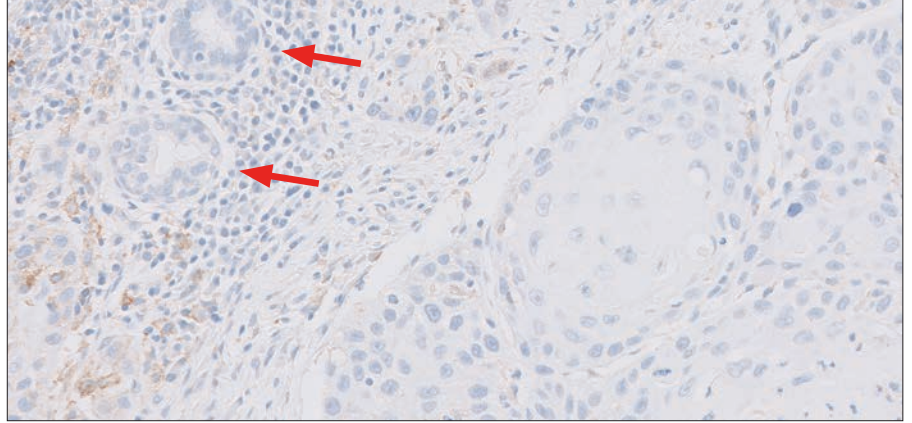


Figure 21: HNSCC specimen stained with PD-L1 primary antibody exhibiting well differentiated, highly cytoplasmic tumor cell pattern. Note the two salivary gland acini (arrows) that should be excluded from the score (20× magnification).

Key Point

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

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 and/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 page 20 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.

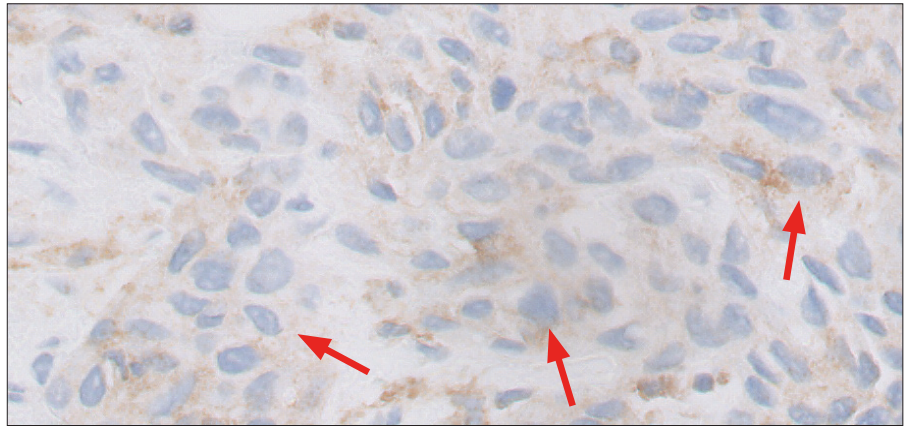


Figure 22: HNSCC specimen stained with PD-L1 primary antibody exhibiting only cytoplasmic staining (arrows) (20× magnification).

Key Point

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

Benign Glands

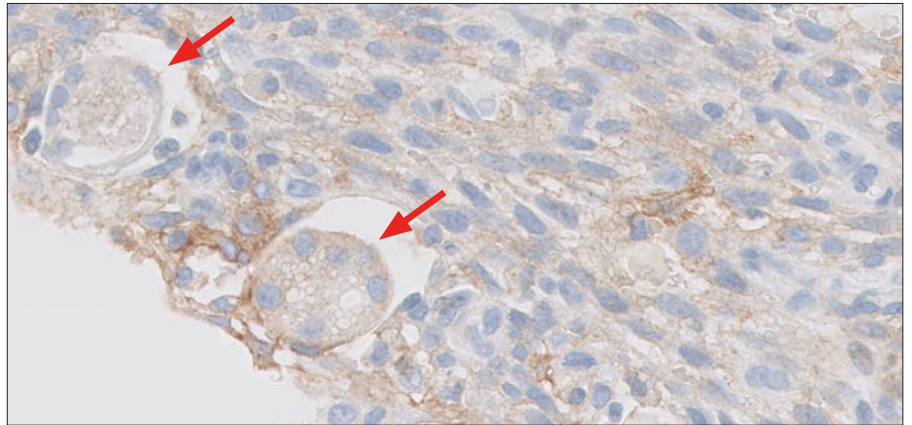


Figure 23: Entrapped salivary gland acini (arrows) (20× magnification).

Key Point

Entrapped salivary gland acini may exhibit PD-L1 staining and should be excluded from the score

Carcinoma In Situ (CIS)

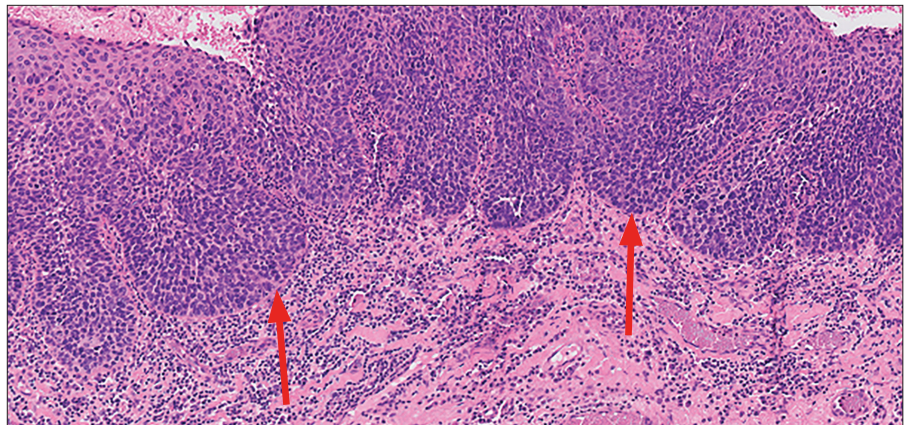


Figure 24a: Hematoxylin and eosin (H&E) section demonstrating carcinoma in situ (CIS) (arrows) (5× magnification).

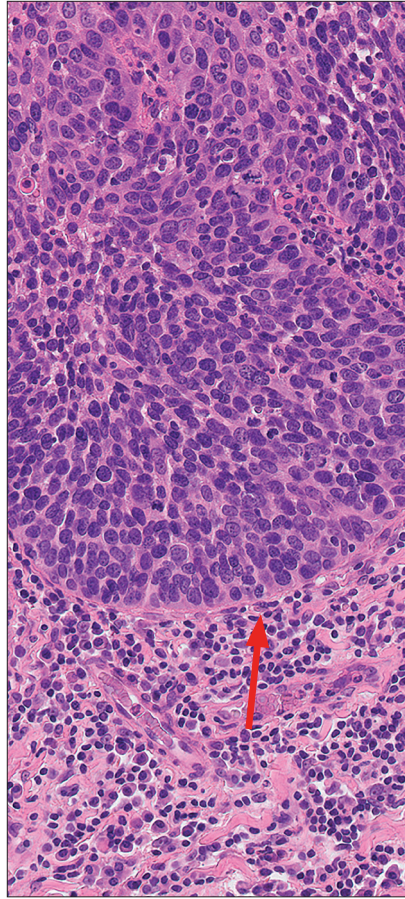


Figure 24b: Hematoxylin and eosin (H&E) section demonstrating carcinoma in situ (CIS) (arrow) (10× magnification).

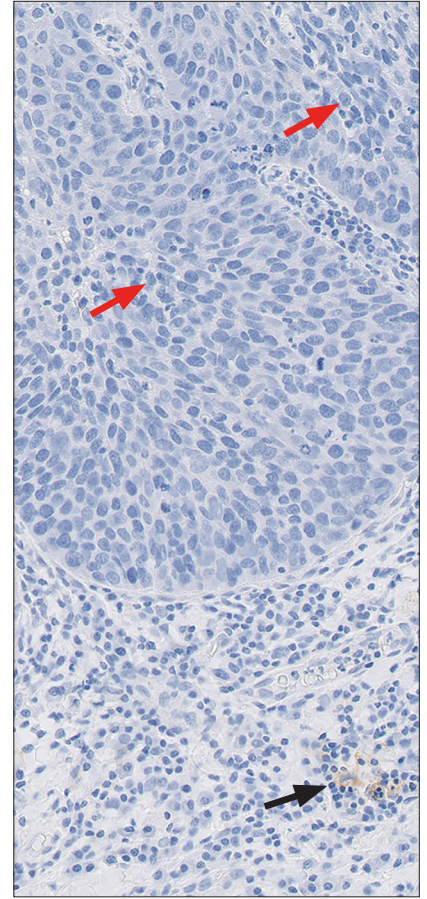


Figure 24c: Any tumor cells that are part of the CIS component should be excluded from the numerator and denominator (red arrows). Any mononuclear inflammatory cells (MICs) (black arrow) associated with the CIS component should be excluded from the numerator (10× magnification).

Key Point

Any tumor cells and MICs associated with the CIS component should be excluded from the score

Normal Tonsil Tissue

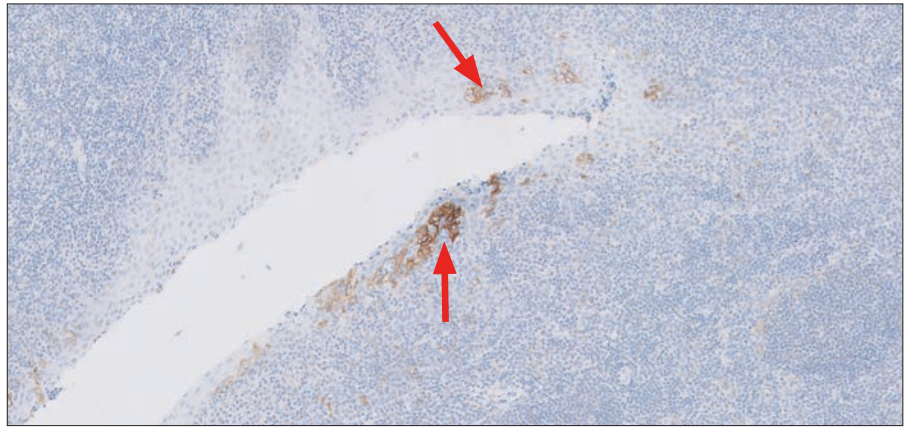


Figure 25: Normal tonsil tissue cells exhibiting PD-L1 staining (arrows) in the crypt epithelium (10× magnification).

Key Point

Normal tonsil tissue can contain cells exhibiting PD-L1 staining in the crypt epithelium and may show considerable cytologic atypia, and should not be mistaken for cancer cells

Stromal Cells



Figure 26: PD-L1 staining on stromal cells (arrows) (20× magnification).

Key Point

Stromal cells exhibiting PD-L1 staining 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 33 for the immune cell inclusion/exclusion 20× rule. PD-L1 staining neutrophils, eosinophils, and plasma cells should be excluded from the score.

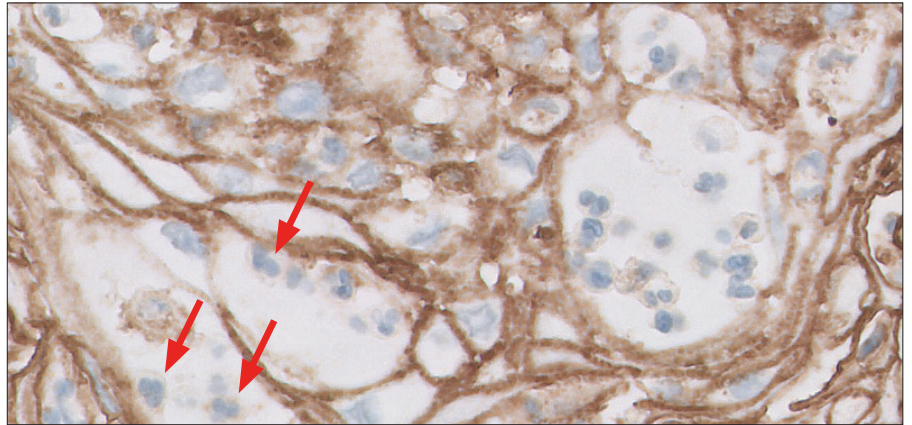


Figure 27a: PD-L1 staining on neutrophils (arrows) (20× magnification).

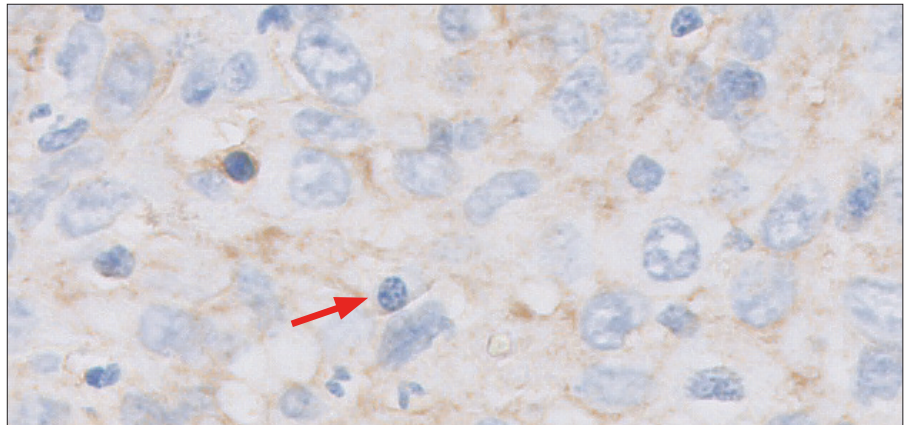


Figure 27b: PD-L1 staining on a plasma cell (arrow) (20× magnification).

Key Point

PD-L1 staining neutrophils, eosinophils, and plasma 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.

Specific Nonscorable Staining

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

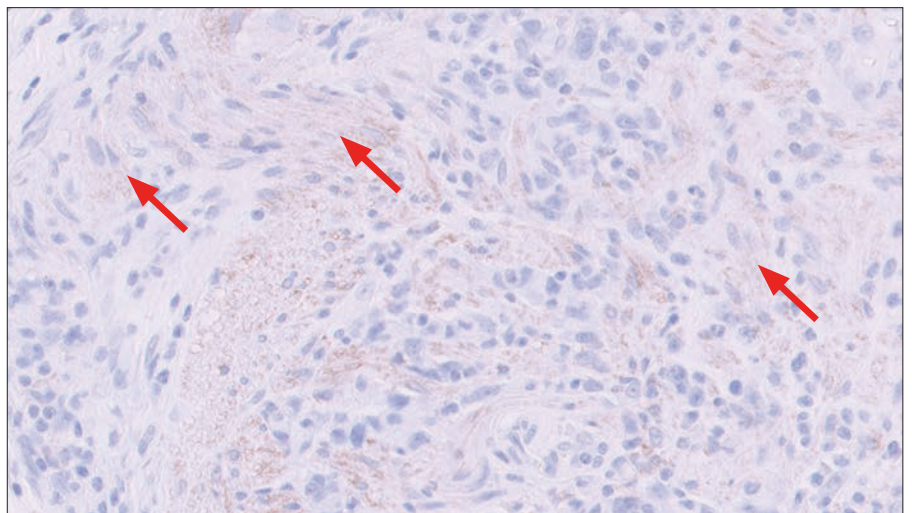
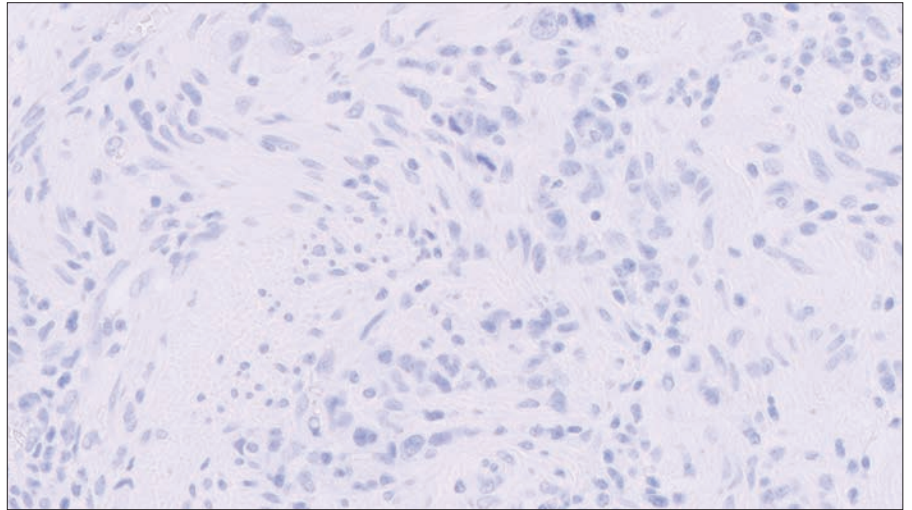


Figure 28a: Gastric or GEJ adenocarcinoma specimen exhibiting $\leq 1+$ intensity specific nonscorable cytoplasmic staining of peripheral nerve cells (arrows). Absence of similar staining in the NCR-stained slide (top) indicates that this cytoplasmic staining in the PD-L1 slide (bottom) is specific, but normal cells such as peripheral nerve cells should be excluded from scoring (20 \times magnification).

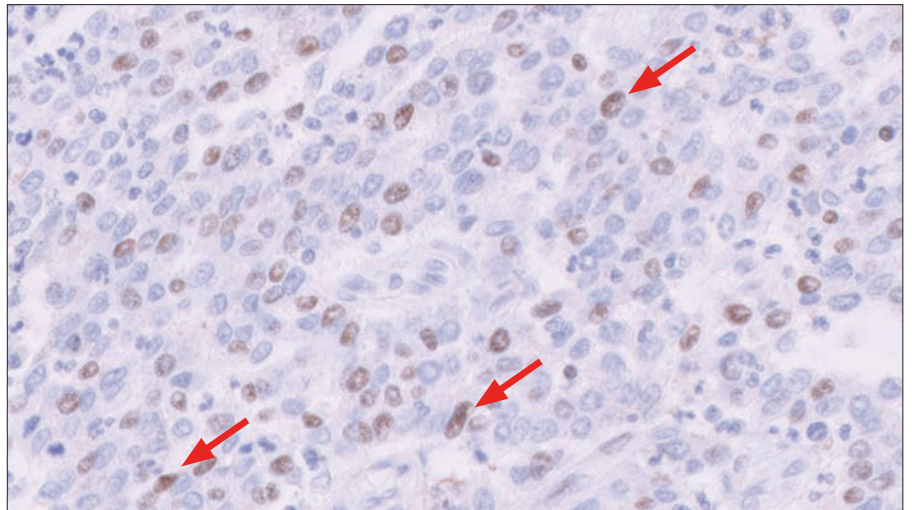
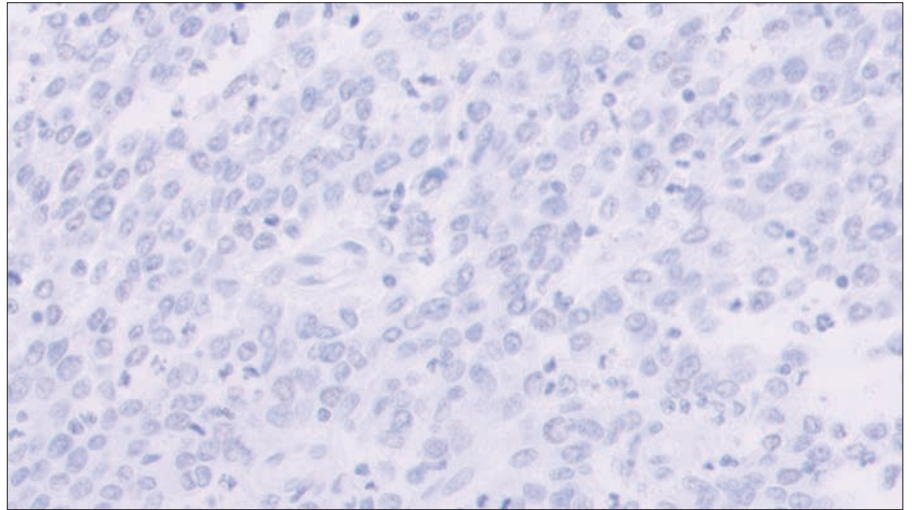


Figure 28b: Gastric or GEJ adenocarcinoma specimen with nuclear staining of tumor cells absent from the NCR-stained slide (top) and present in the PD-L1 stained slide (arrows) (bottom), indicating that this nuclear staining is specific staining. Since the nucleus is a cell compartment that is not scorable, and the nuclear reactivity does not interfere with evaluation of membrane staining on tumor cells, the PD-L1 slide should be considered evaluable (20× magnification).

Nonspecific Staining

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

- Pre-analytic fixation and processing of the specimen, including use of fixatives other than neutral buffered formalin (not recommended)
- Incomplete removal of paraffin from the section
- Incomplete rinsing of reagents from slides during staining
- Drying of slides; ensure slides remain wet with buffer while loading onto Autostainer Link 48, prior to initiating run and during the staining procedure (only relevant for Code SK006)
- Cross-reactivity of the secondary antibody in the detection system
- Reagent trapping (tissue folding; tissue drying; hydrophobic or ionic interactions with ‘sticky’ tissues or substances such as cartilage, muscle fibers, dense fibrosis, mucin, necrotic debris)

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

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

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

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

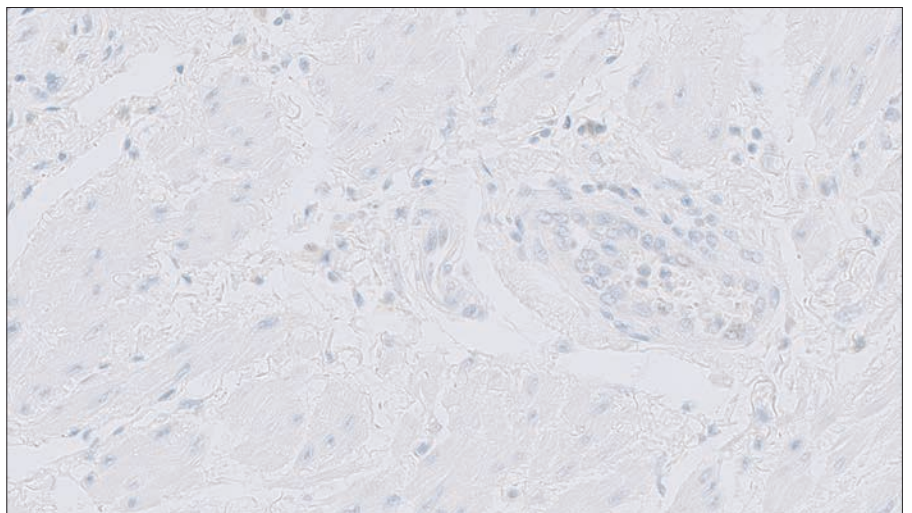
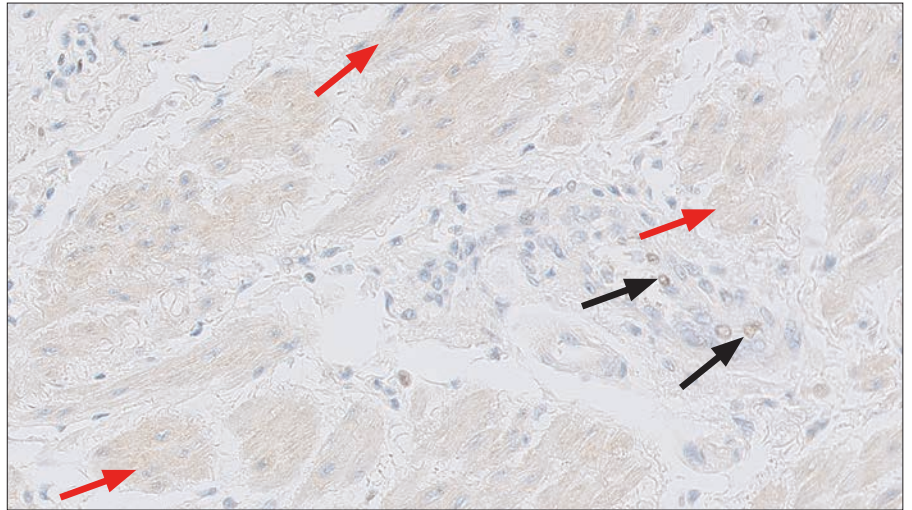


Figure 29a: Esophageal adenocarcinoma specimen stained with NCR (top) exhibiting acceptable ($\leq 1+$ intensity) nonspecific staining on smooth muscle cells (red arrows) and occasional endothelial cell nuclei (black arrows). Note that in this case, the nonspecific staining is not also present in the PD-L1 slide (bottom) (20 \times magnification).

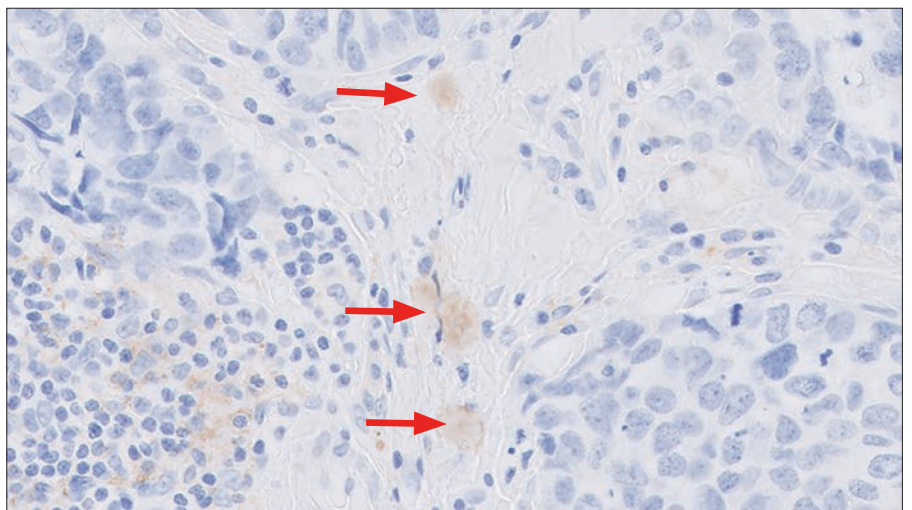


Figure 29b: TNBC specimen stained with PD-L1 primary antibody exhibiting acceptable ($\leq 1+$ intensity) nonspecific DAB staining (arrows)(20 \times magnification).

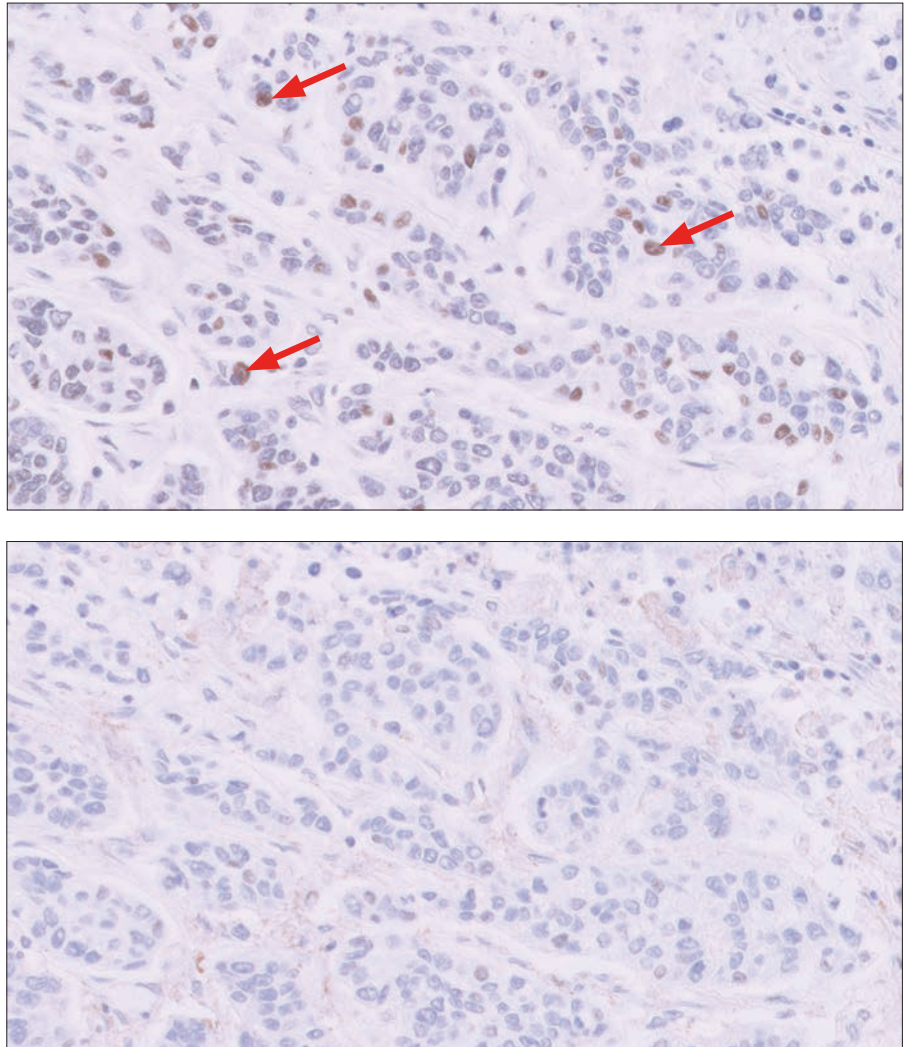


Figure 29c: Gastric or GEJ adenocarcinoma specimen exhibiting > 1+ nonspecific nuclear staining in the NCR-(top) stained slide (arrows) that is not also present in the PD-L1 (bottom) stained slide. As the acceptance criteria for nonspecific staining, including nuclear staining in the NCR and PD-L1 slides or NCR slide alone is \leq 1+ intensity, the PD-L1 stained slide in this case should be considered non-evaluable (20 \times magnification).

Key Point

All specimens must have \leq 1+ nonspecific staining, including nuclear staining within scorable tumor regions

Edge Artifact

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

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

Both factors can lead to accentuation of staining at the periphery of the section, and minimal staining or nonstaining in the central portion. 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.

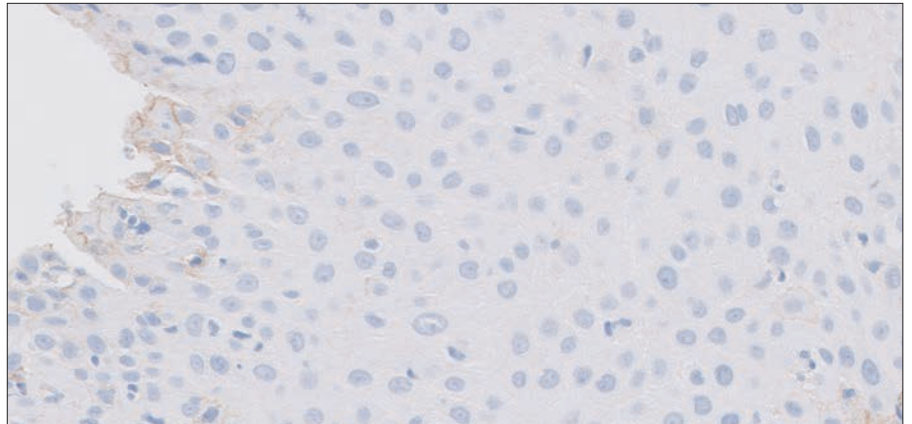


Figure 30: Edge staining should be excluded from the score (20× magnification). **Note:** Squamous cell carcinoma from the cervix is depicted.

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.

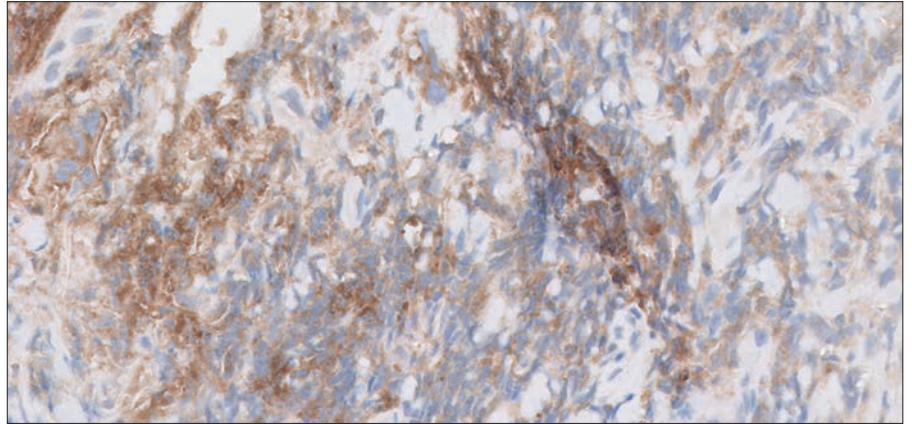


Figure 31: HNSCC specimen stained with PD-L1 primary antibody exhibiting crush artifact; crush artifact should be excluded from the score (20× magnification).

Key Point

Scoring of crush artifact should be avoided

Necrosis

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

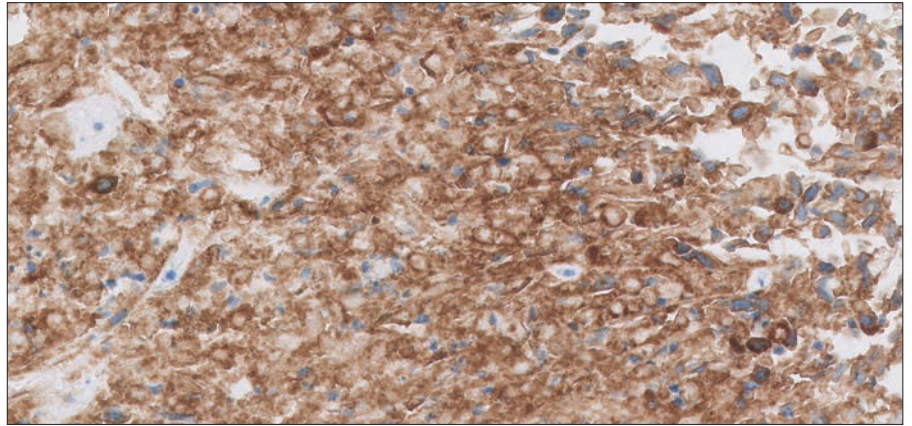


Figure 32: HNSCC specimen stained with PD-L1 primary antibody exhibiting staining of necrosis; necrosis staining should be excluded from the score (20× magnification).

Key Point

Scoring of necrotic areas should be excluded from the CPS calculation

PD-L1 IHC 22C3 pharmDx CPS Case Examples

CPS < 1 Case Examples

Scores for single 20× field case examples are provided solely as examples to demonstrate a range of CPS scores. When testing samples for PD-L1 expression using PD-L1 IHC 22C3 pharmDx, the entire sample should be scored to determine the patient CPS result.

Case 1: CPS 0

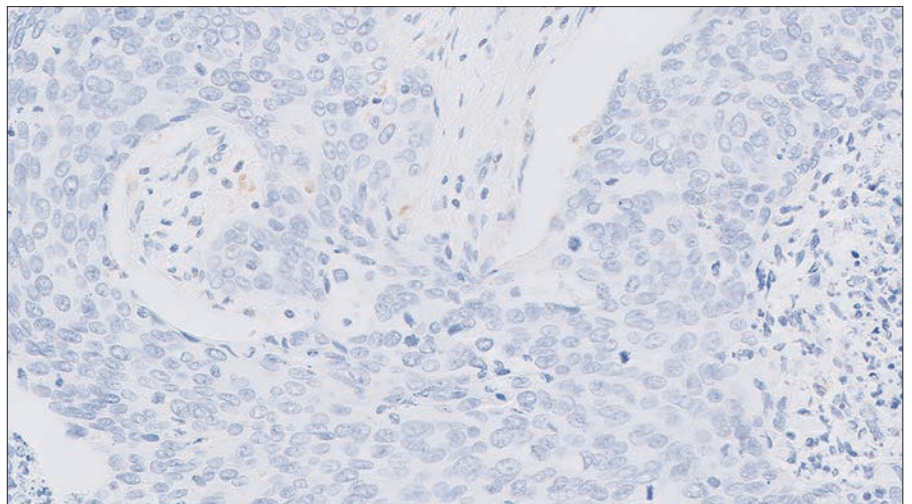


Figure 33: HNSCC specimen stained with PD-L1 antibody exhibiting CPS 0 (20× magnification).

Case 2: CPS 0

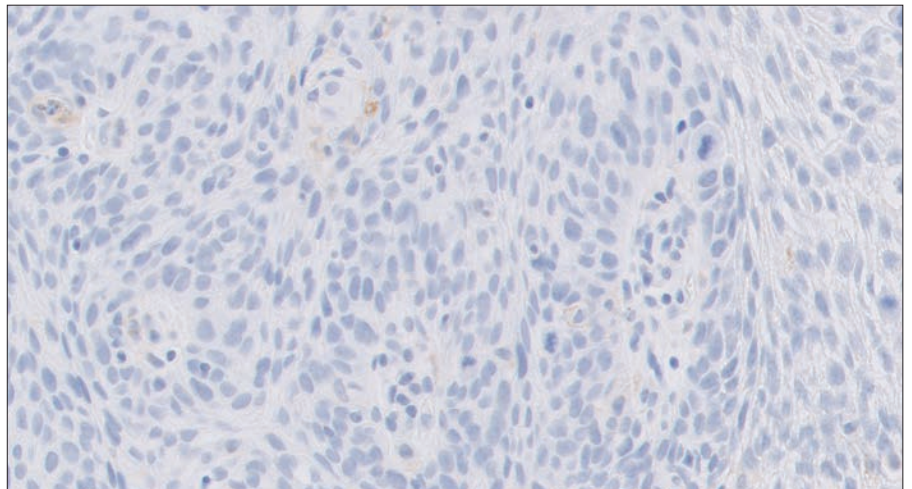


Figure 34: HNSCC specimen stained with PD-L1 antibody exhibiting CPS 0 (20× magnification).

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)

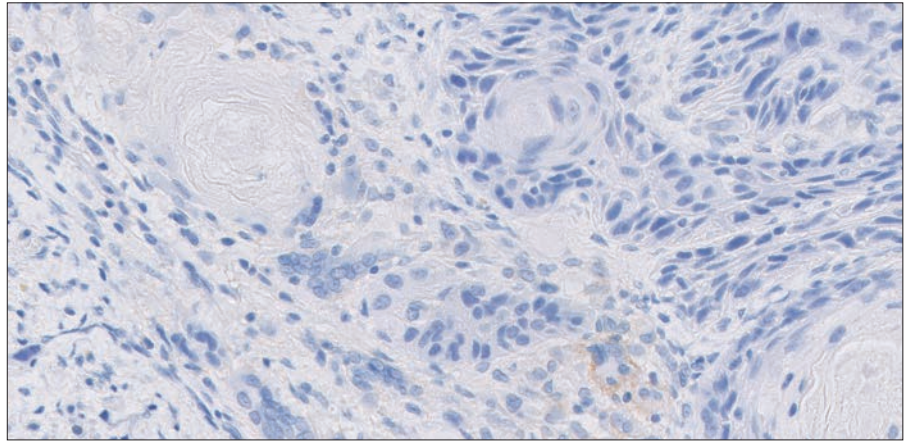


Figure 35: HNSCC specimen stained with PD-L1 antibody exhibiting a CPS of 1, however any numerical CPS between 1–2 could be assigned to this image (20× magnification).

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

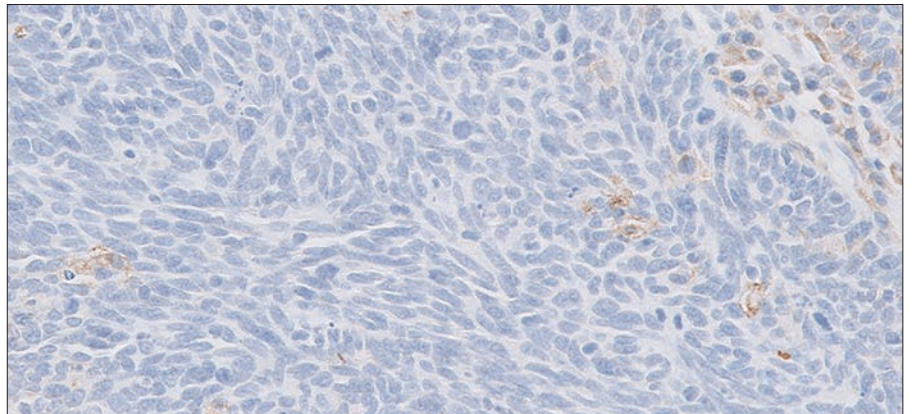


Figure 36: HNSCC specimen stained with PD-L1 antibody exhibiting a CPS of 2, however any numerical CPS between 1–3 could be assigned to this image (20× magnification).

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

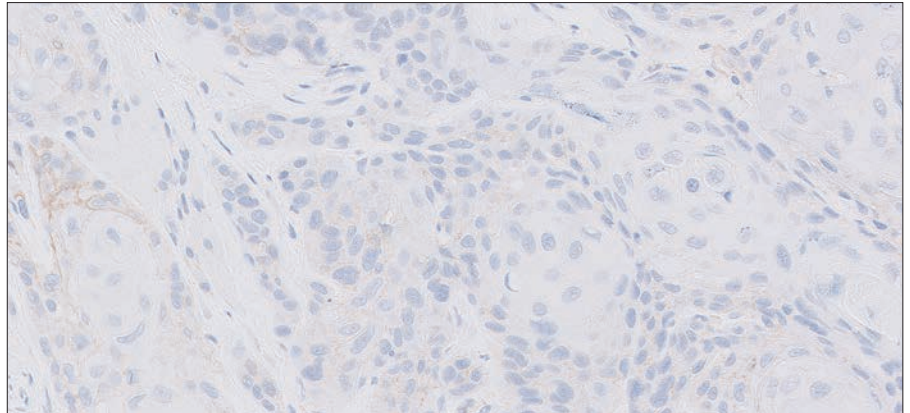


Figure 37: HNSCC specimen stained with PD-L1 antibody exhibiting a CPS of 7, however any numerical CPS between 5–9 could be assigned to this image (20× magnification).

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

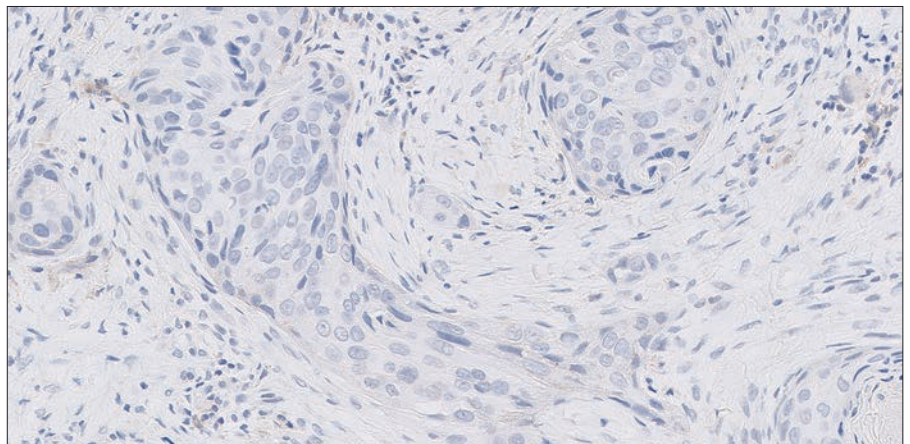


Figure 38: HNSCC specimen stained with PD-L1 antibody exhibiting a CPS of 5, however any numerical CPS between 3–7 could be assigned to this image (20× magnification).

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

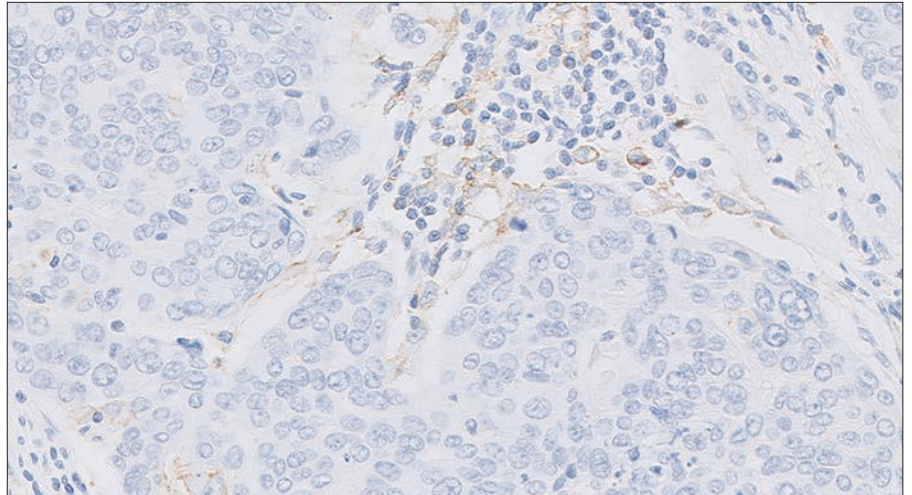


Figure 39: HNSCC specimen stained with PD-L1 antibody exhibiting a CPS of 8, however any numerical CPS between 6–10 could be assigned to this image (20× magnification).

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

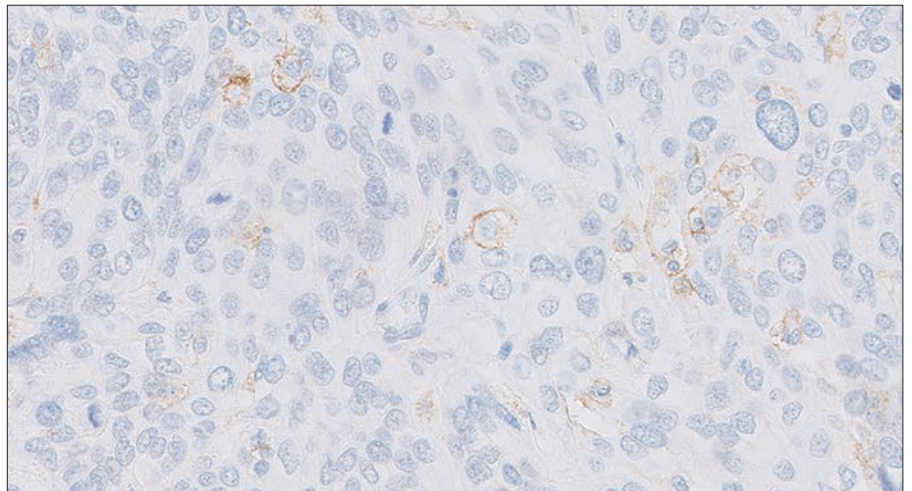


Figure 40: HNSCC specimen stained with PD-L1 antibody exhibiting a CPS of 6, however any numerical CPS between 4–8 could be assigned to this image (20× magnification).

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

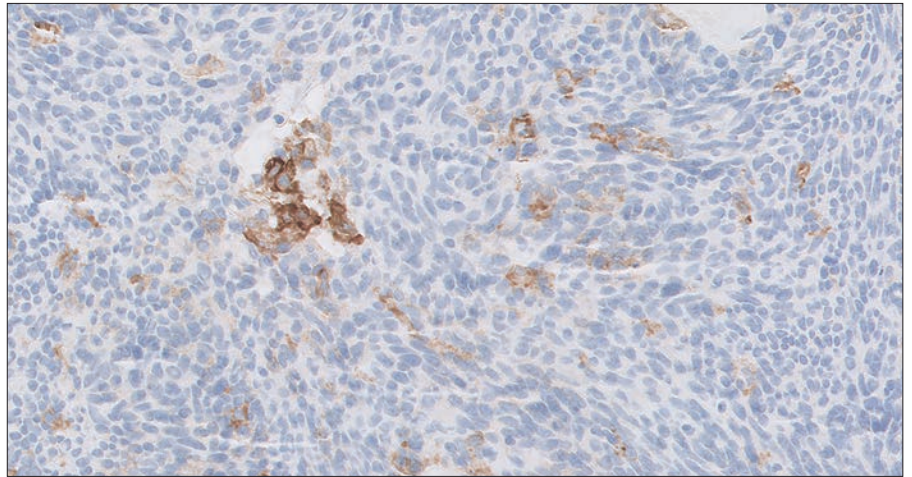


Figure 41: HNSCC specimen stained with PD-L1 antibody exhibiting a CPS of 4, however any numerical CPS between 2–6 could be assigned to this image (20× magnification).

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

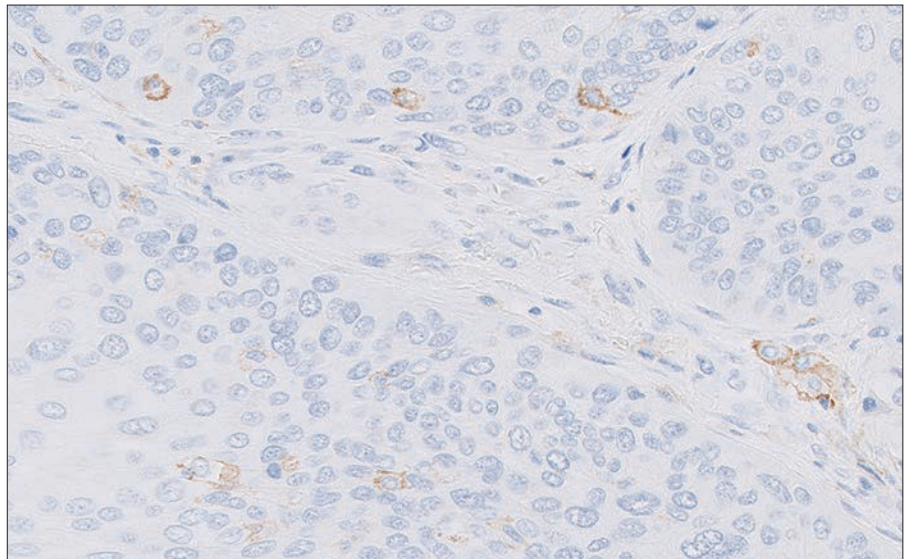


Figure 42: HNSCC specimen stained with PD-L1 antibody exhibiting a CPS of 4, however any numerical CPS between 2–6 could be assigned to this image (20× magnification).

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

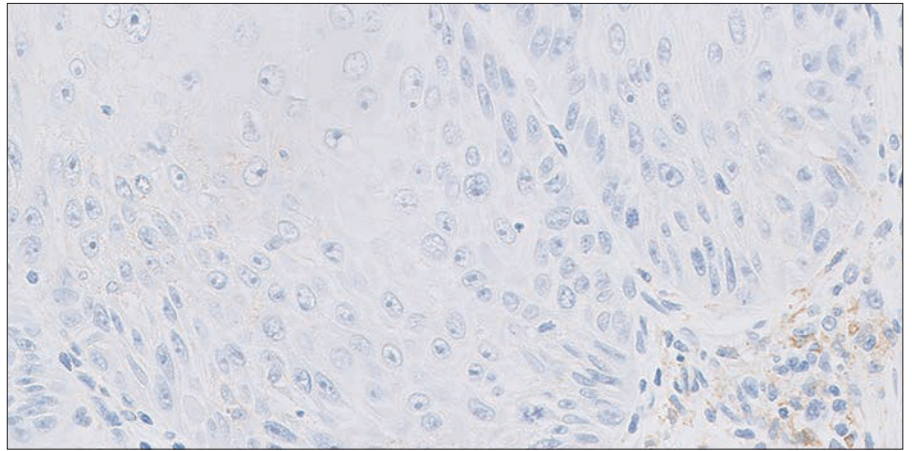


Figure 43: HNSCC specimen stained with PD-L1 antibody exhibiting a CPS of 6, however any numerical CPS between 4–8 could be assigned to this image (20× magnification).

CPS Range of Greater Than 10 but Less Than or Equal to 30

Challenging Case 10: CPS Range Greater Than 10 but Less Than or Equal to 30

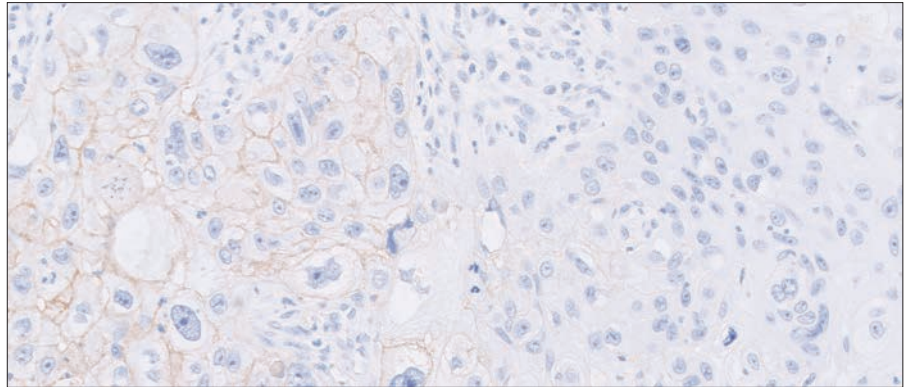


Figure 44: HNSCC specimen stained with PD-L1 antibody exhibiting a CPS of 22, however any numerical CPS between 20–25 could be assigned to this image (20× magnification).

Challenging Case 11: CPS Range Greater Than 10 but Less Than or Equal to 30

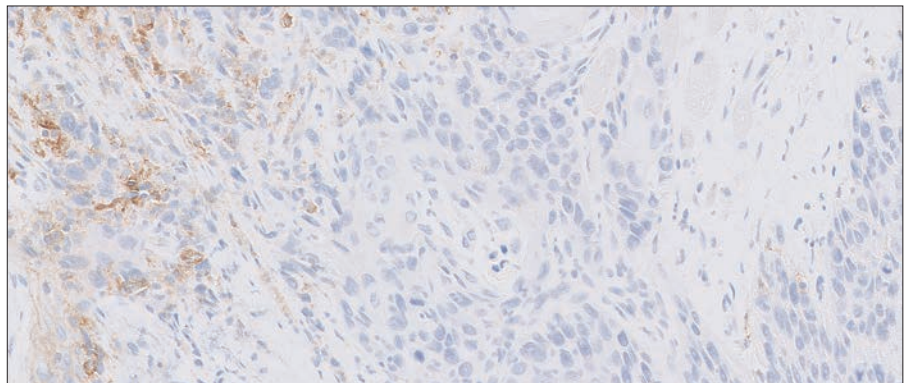


Figure 45: HNSCC specimen stained with PD-L1 antibody exhibiting a CPS of 25, however any numerical CPS between 20–30 could be assigned to this image (20× magnification).

Challenging Case 12: CPS Range Greater Than 10 but Less Than or Equal to 30

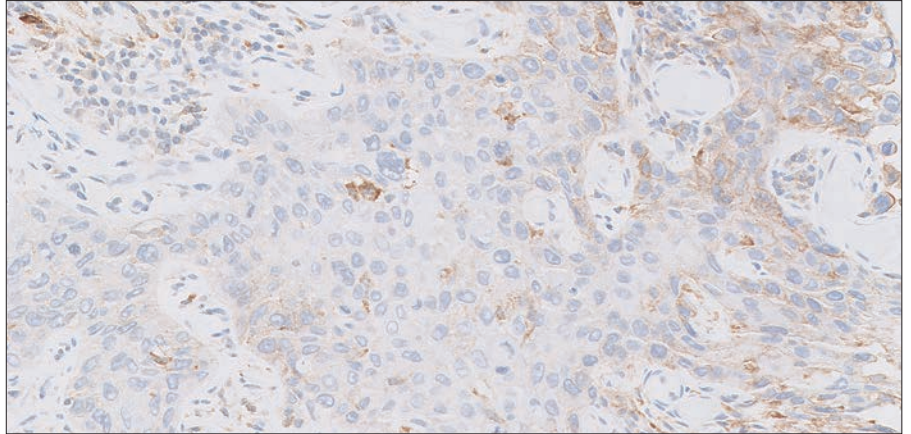


Figure 46: HNSCC specimen stained with PD-L1 antibody exhibiting a CPS of 25, however any numerical CPS between 20–30 could be assigned to this image. PD-L1 staining plasma cells in the upper left corner of the field should be excluded from scoring (20× magnification).

Challenging Case 13: CPS Range Greater Than 10 but Less Than or Equal to 30

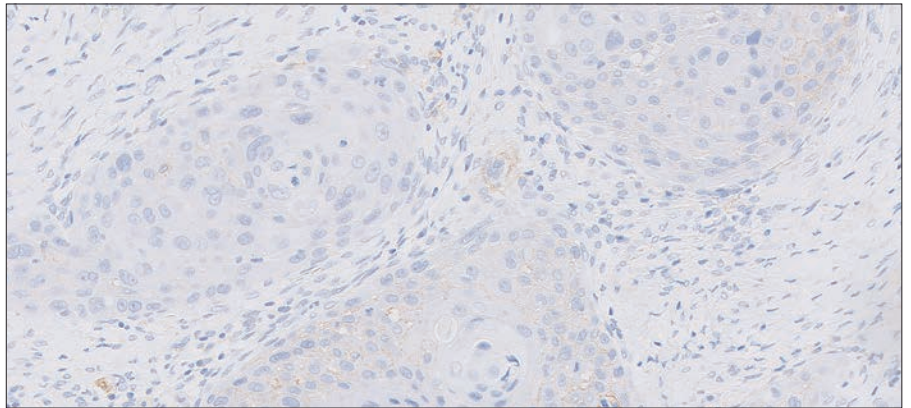


Figure 47: HNSCC specimen stained with PD-L1 antibody exhibiting a CPS of 22, however any numerical CPS between 20–25 could be assigned to this image (20× magnification).

CPS \geq 20 Case Examples

Case 3: CPS \geq 20

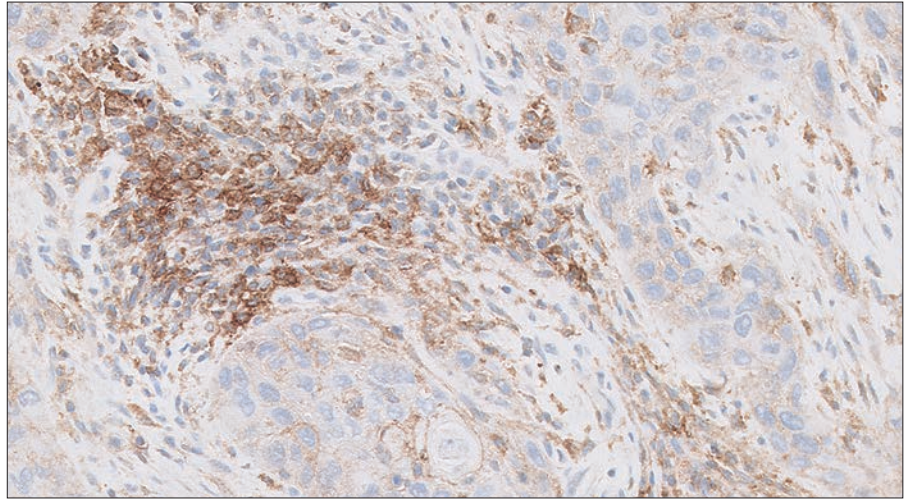


Figure 48: HNSCC specimen stained with PD-L1 antibody exhibiting a CPS of 100 (20 \times magnification).

Case 4: CPS \geq 20

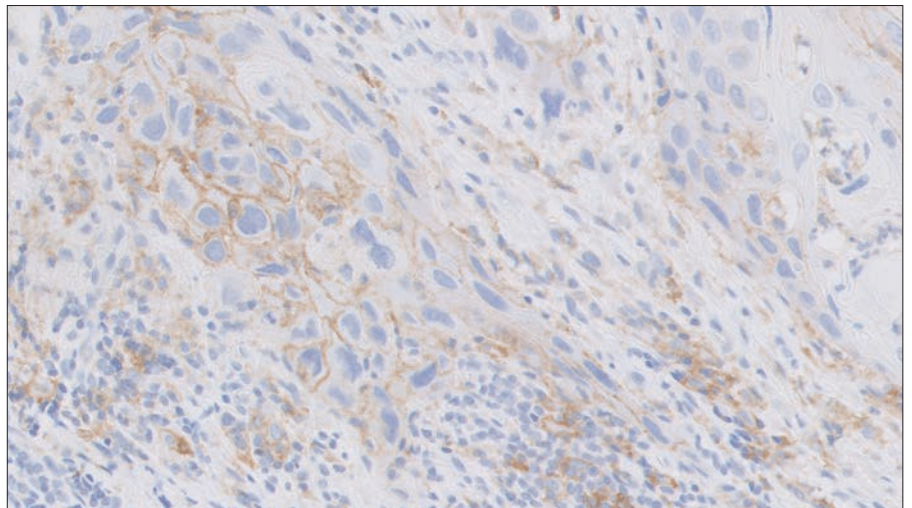


Figure 49: HNSCC specimen stained with PD-L1 antibody exhibiting a CPS of 100 (20 \times magnification).

Case 5: CPS \geq 20

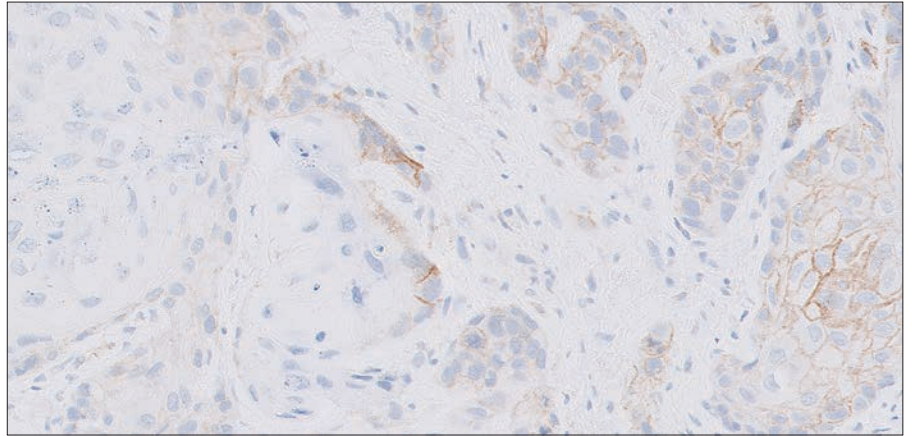


Figure 50: HNSCC specimen stained with PD-L1 antibody exhibiting a CPS of 50, however any numerical CPS between 45–55 could be assigned to this image (20 \times magnification).

Case 6: CPS \geq 20

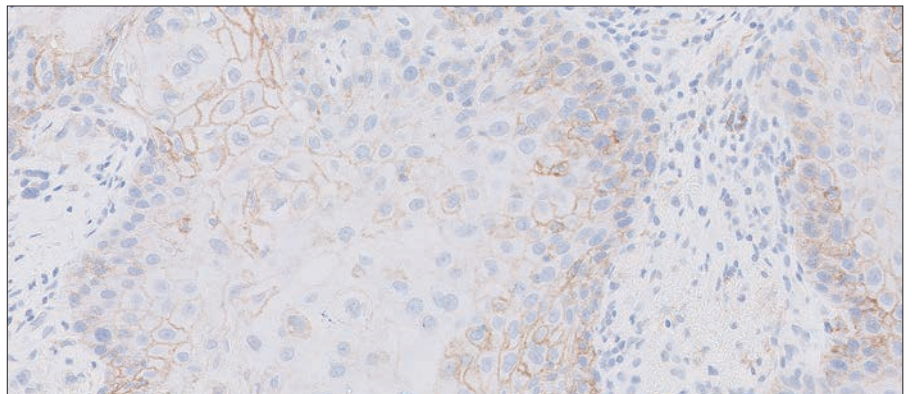


Figure 51: HNSCC specimen stained with PD-L1 antibody exhibiting a CPS of 55, however any numerical CPS between 50–60 could be assigned to this image (20 \times magnification).

Control Cell Line (CCL) Appendix

Passing CCL

Passing PD-L1 Negative CCL

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

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

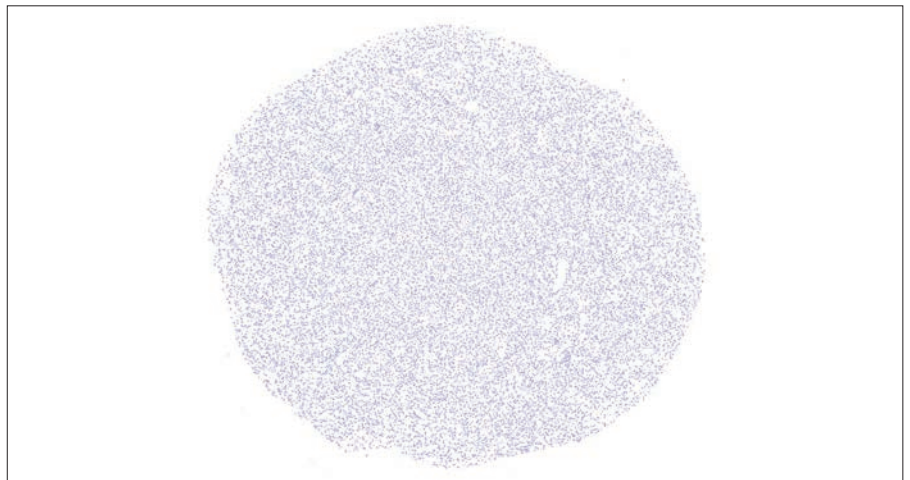


Figure 52: Ideal MCF-7 cell pellet (2x magnification).

Passing PD-L1 Positive CCL

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



Figure 53: Ideal NCI-H226 cell pellet (2x magnification).

Borderline Passing CCL

Borderline Passing vs. Passing PD-L1 Positive CCL

Borderline Passing PD-L1 positive CCL



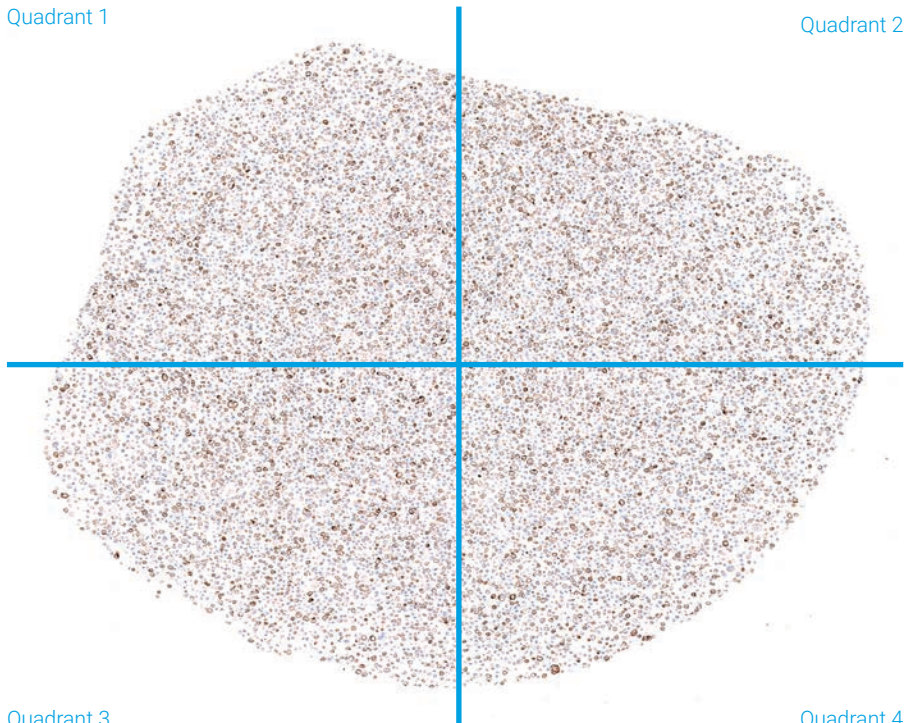
Figure 54: NCI-H226 cell pellet (2x magnification).

Evaluation Strategy for Borderline Passing PD-L1 Positive CCL

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

Quadrant 1

Quadrant 2



Quadrant 3

Quadrant 4

Quadrant 1

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

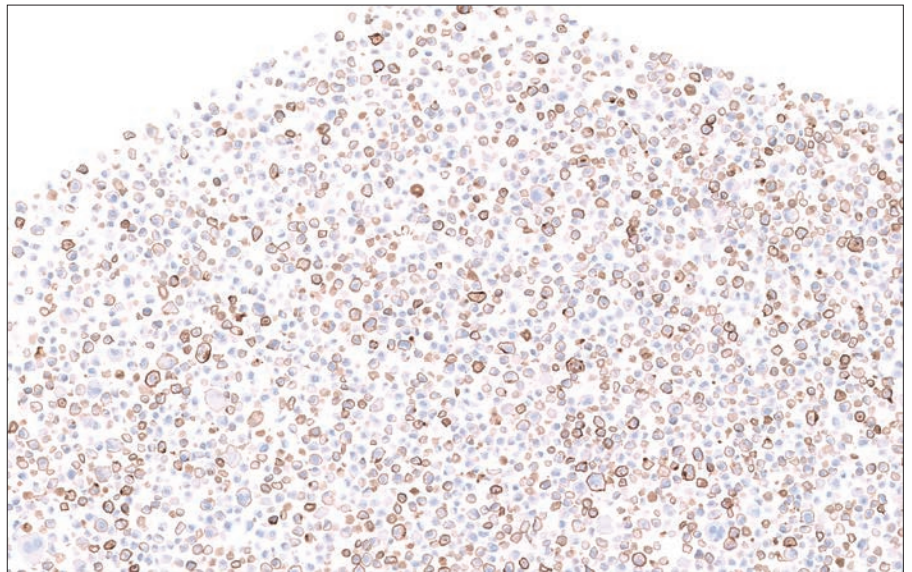


Figure 55: NCI-H226 cell pellet (5 \times magnification).

Quadrant 2

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

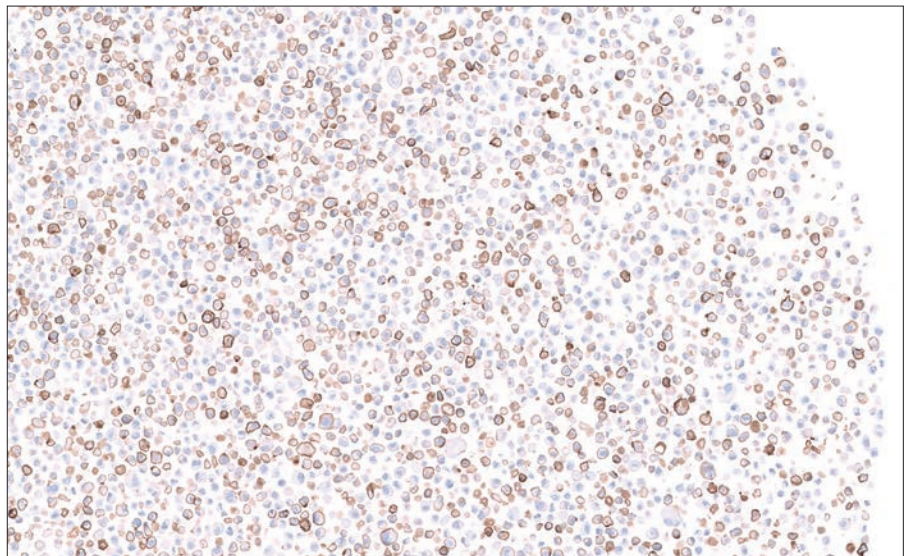


Figure 56: NCI-H226 cell pellet (5 \times magnification).

Quadrant 3

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

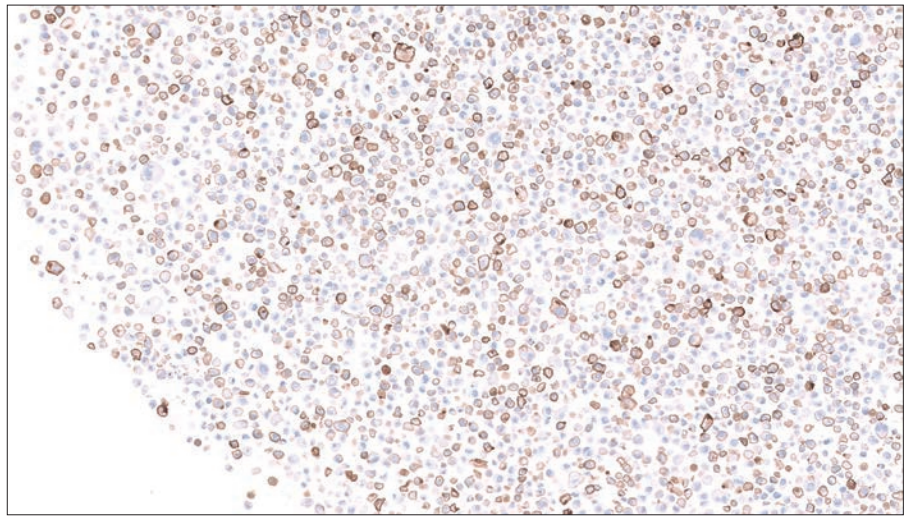


Figure 57: NCI-H226 cell pellet (5× magnification).

Quadrant 4

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

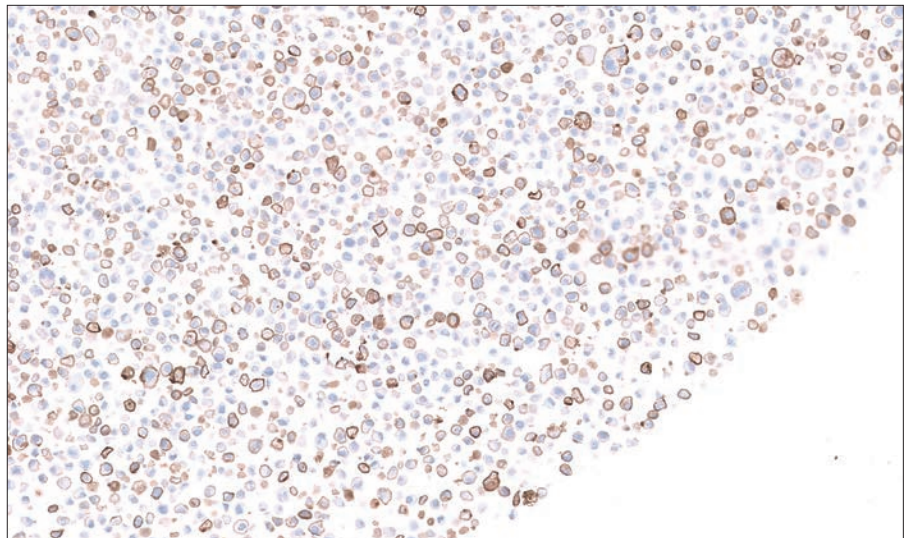
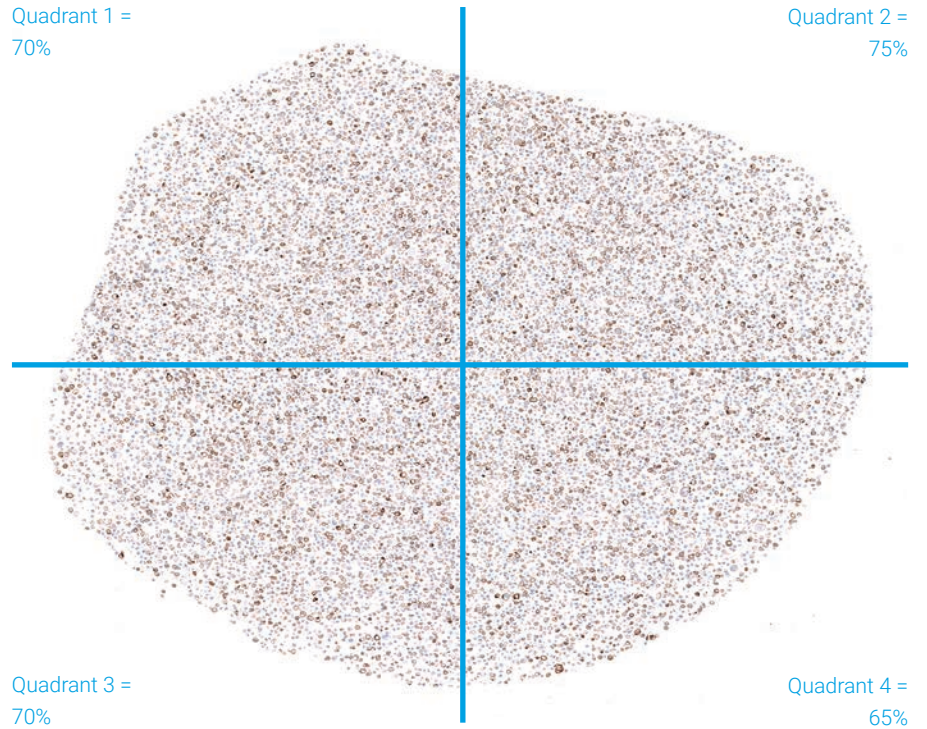


Figure 58: NCI-H226 cell pellet (5× magnification).

Calculation

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



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

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

NCI 226 positive control cell pellet meets acceptance criteria.

Failed CCL

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

Passing PD-L1 negative CCL

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

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

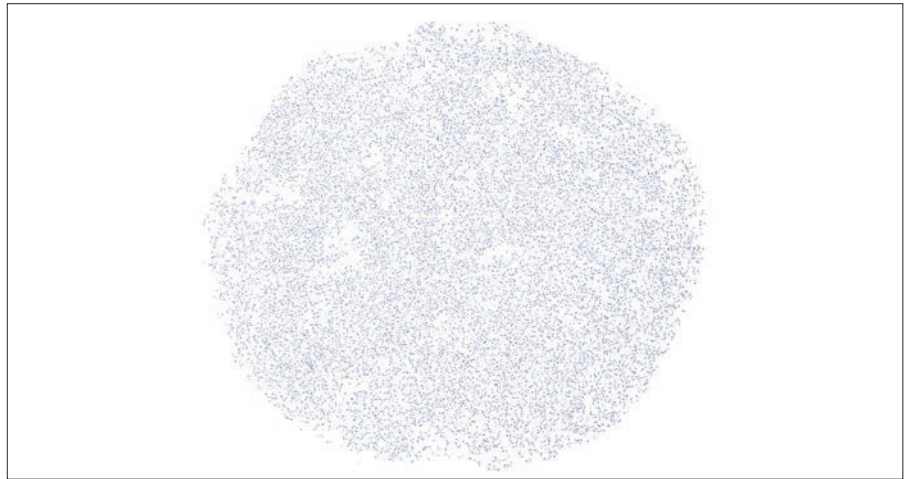


Figure 59: MCF-7 cell pellet (2x magnification).

Failed PD-L1 positive CCL

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

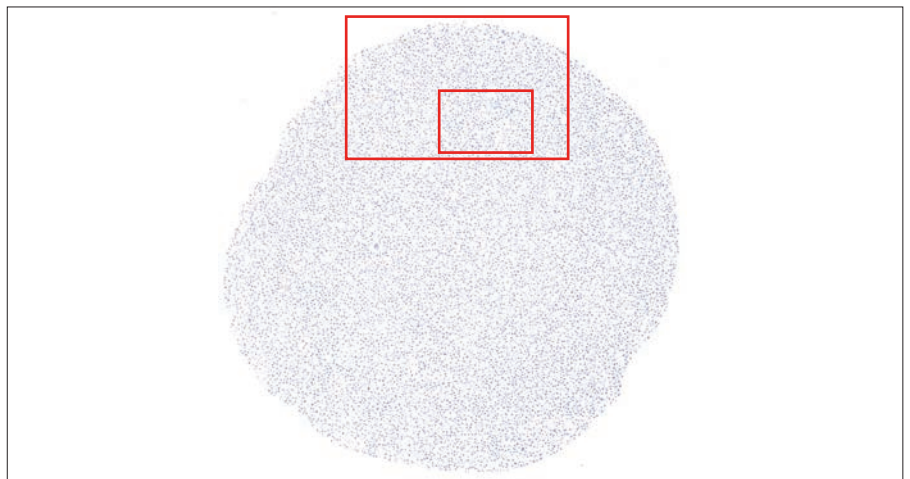


Figure 60: NCI-H226 cell pellet (2x magnification).

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

Failed PD-L1 positive CCL (10x)

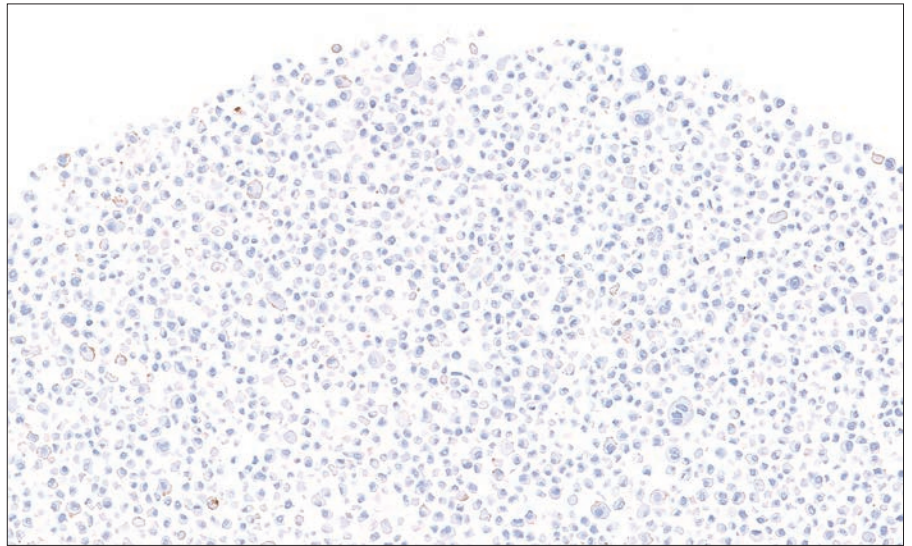


Figure 61: NCI-H226 cell pellet (10x magnification).

Failed PD-L1 positive CCL (20x)

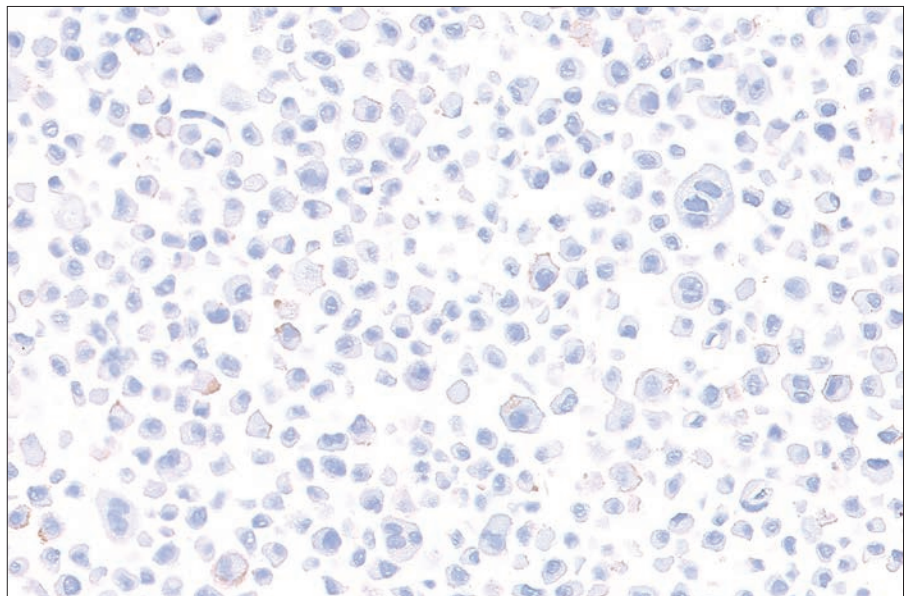


Figure 62: NCI-H226 cell pellet (20x magnification).

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

Passing PD-L1 negative CCL

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

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

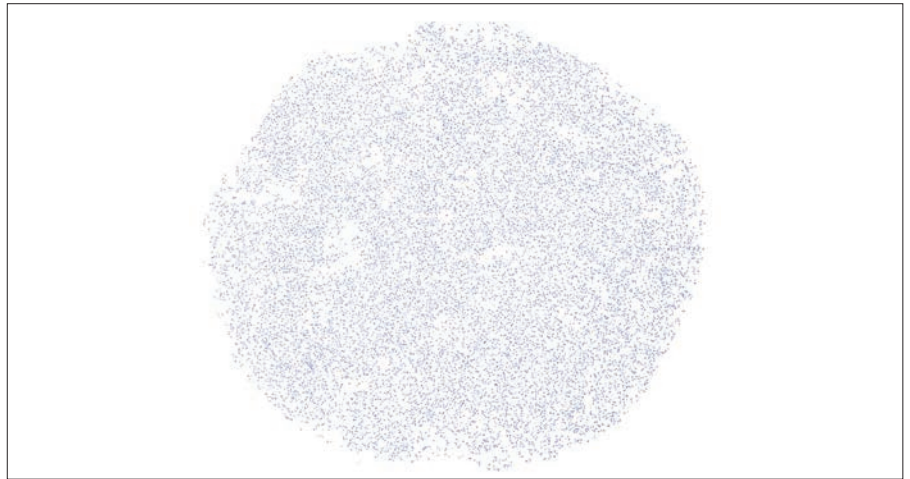


Figure 63: MCF-7 cell pellet (2× magnification).

Failed PD-L1 positive CCL

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



Figure 64: NCI-H226 cell pellet (2× magnification).

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

Failed PD-L1 positive CCL (10x)

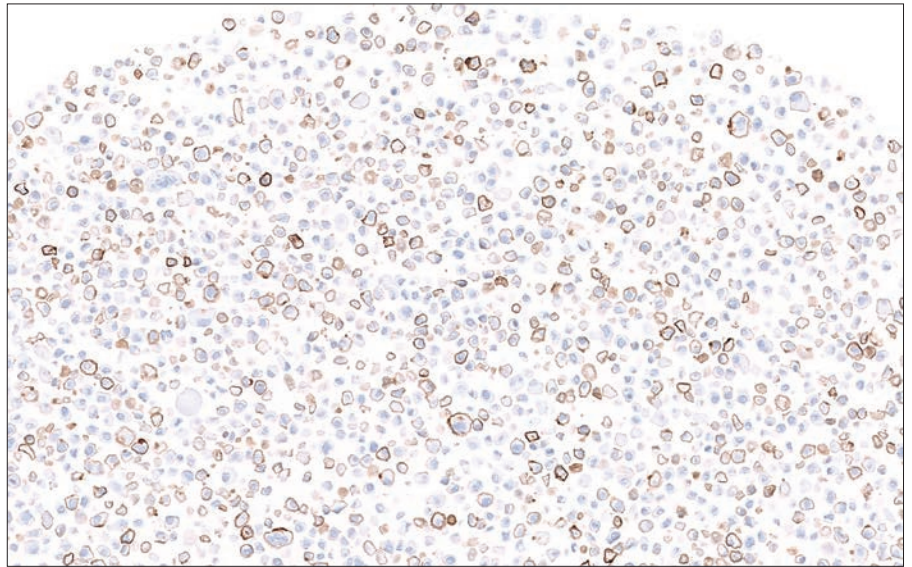


Figure 65: NCI-H226 cell pellet (10x magnification).

Failed PD-L1 positive CCL (20x)

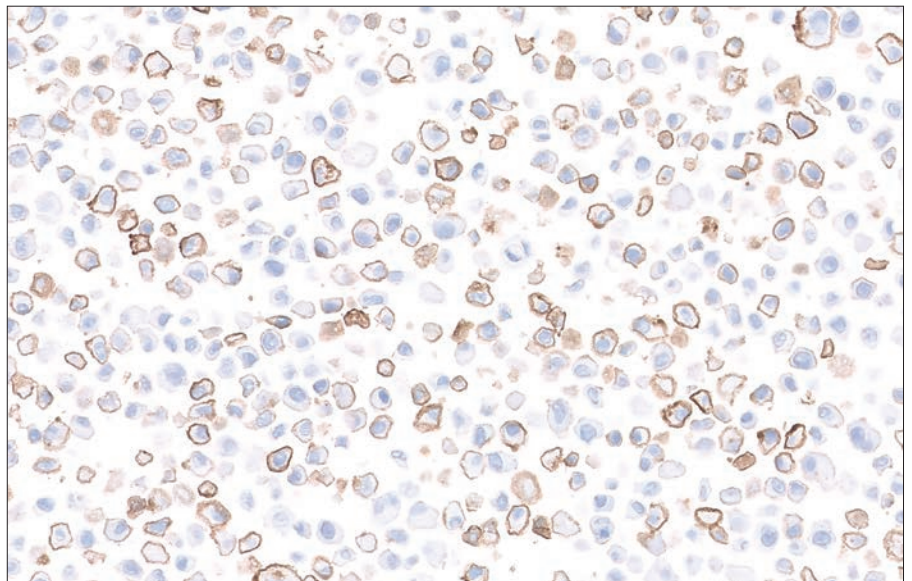


Figure 66: NCI-H226 cell pellet (20x magnification).

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

Passing PD-L1 positive CCL

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



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

Failed PD-L1 negative CCL

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

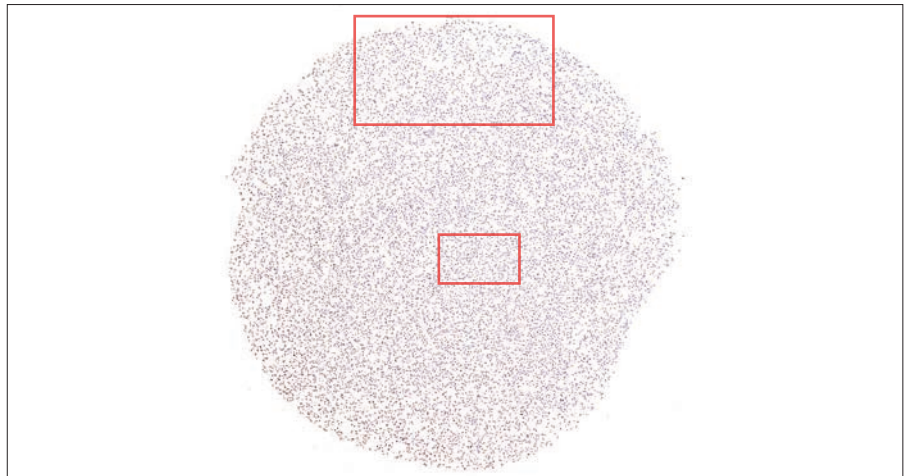


Figure 68: MCF-7 cell pellet (2x magnification).

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

Failed PD-L1 negative CCL (10×)

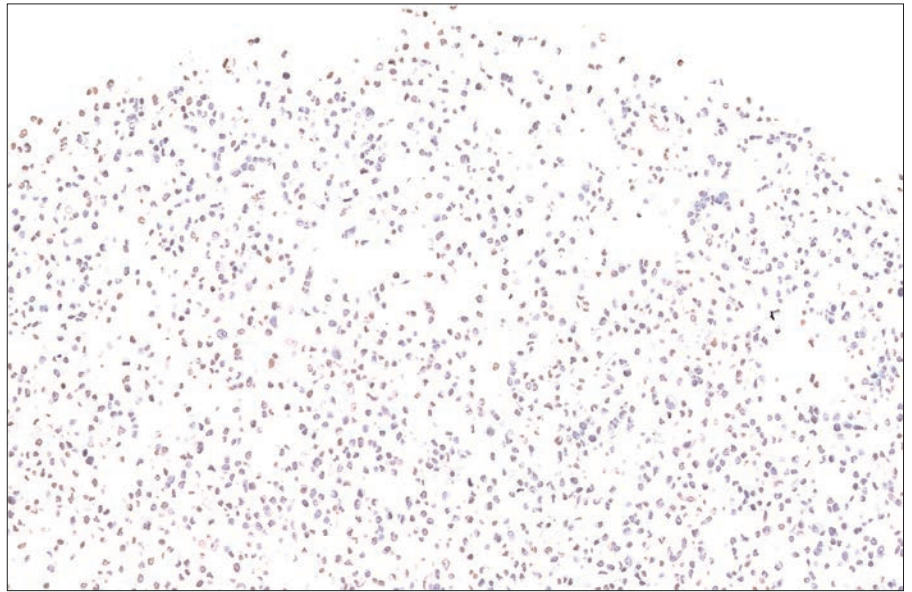


Figure 69: MCF-7 cell pellet (10× magnification).

Failed PD-L1 negative CCL (20×)

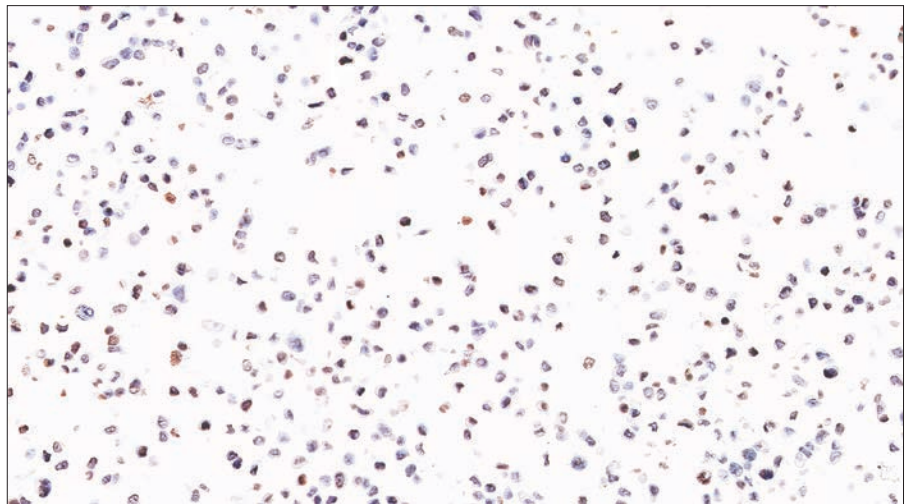


Figure 70: MCF-7 cell pellet (20× magnification).

Troubleshooting Guide

Troubleshooting Guidelines for PD-L1 IHC 22C3 pharmDx

Refer to the current IFU in your country for PD-L1 IHC 22C3 pharmDx, Code SK006 or Code GE006 troubleshooting guidelines.

References

- Keytruda [package insert]. Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA; 2025.
- PD-L1 IHC 22C3 pharmDx, Code SK006 [package insert]. Carpinteria, CA: Dako, Agilent Pathology Solutions; 2025.
- PD-L1 IHC 22C3 pharmDx, Code GE006 [package insert]. Carpinteria, CA: Dako, Agilent Pathology Solutions; 2025.
- Data on file. Agilent Technologies, Inc.

www.agilent.com

Refer to the country-specific PD-L1 IHC 22C3 pharmDx, Code SK006 or Code GE006 Instructions for Use (IFU) for the detailed intended use statement specifying indications and expression level cutoff values.

D54734_3.00

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Published in the USA, February 9, 2026
29324 2026FEB02

