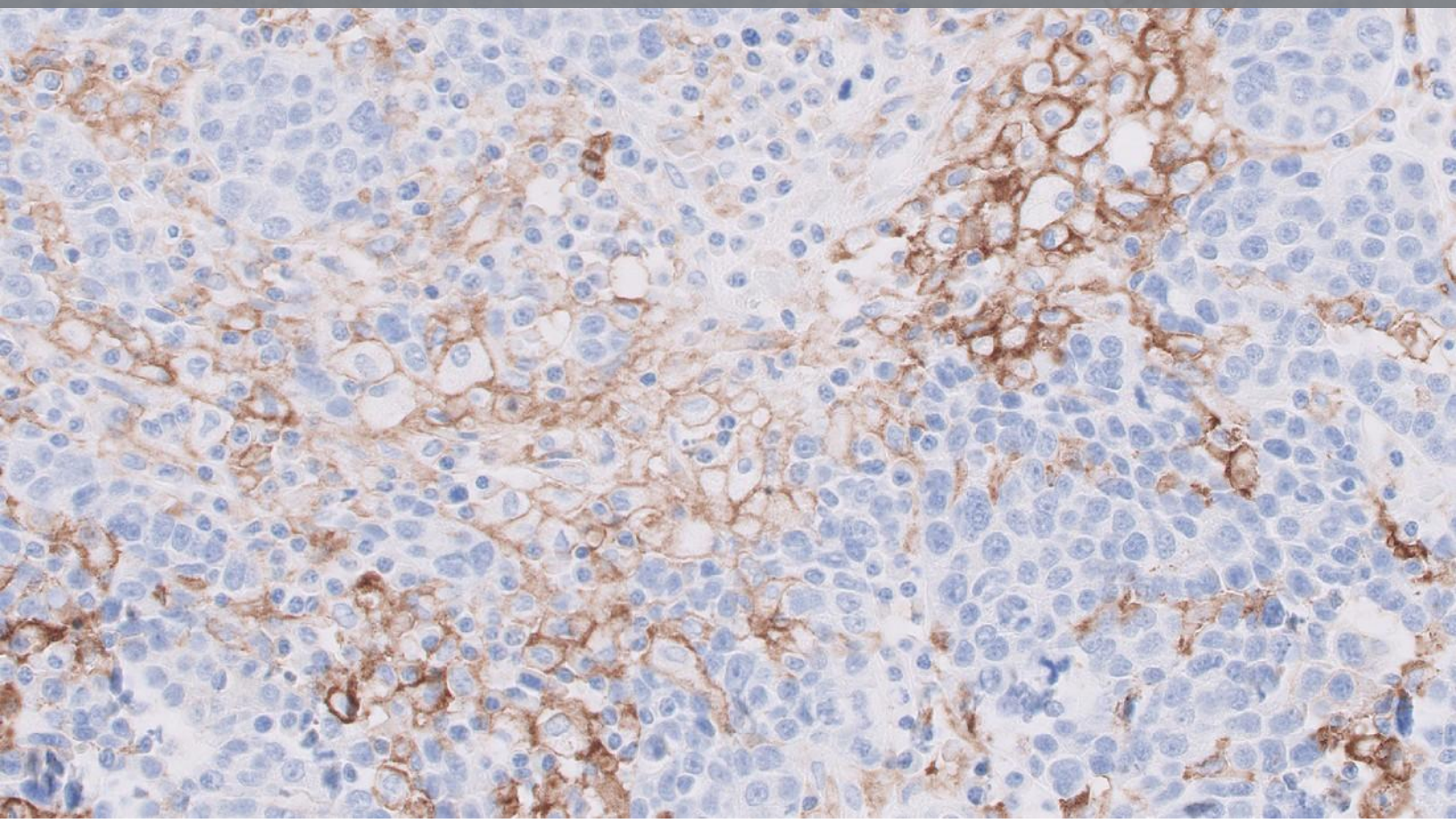
PD-L1 IHC 22C3 pharmDx, Code SK006 Interpretation Manual – Epithelial Ovarian, Fallopian Tube, and Primary Peritoneal Carcinoma (EOC)

Agilent Dako



Agilent

Trusted Answers

This Interpretation Manual can be used with PD-L1 IHC 22C3 pharmDx, Code SK006, for use with Autostainer Link 48 and PT Link.

For countries outside of the United States, see the local KEYTRUDA® (pembrolizumab) product label for approved indications and PD-L1 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) epithelial ovarian, fallopian tube, or primary peritoneal carcinoma (EOC) tissue using EnVision FLEX visualization system on Autostainer Link 48.

EOC

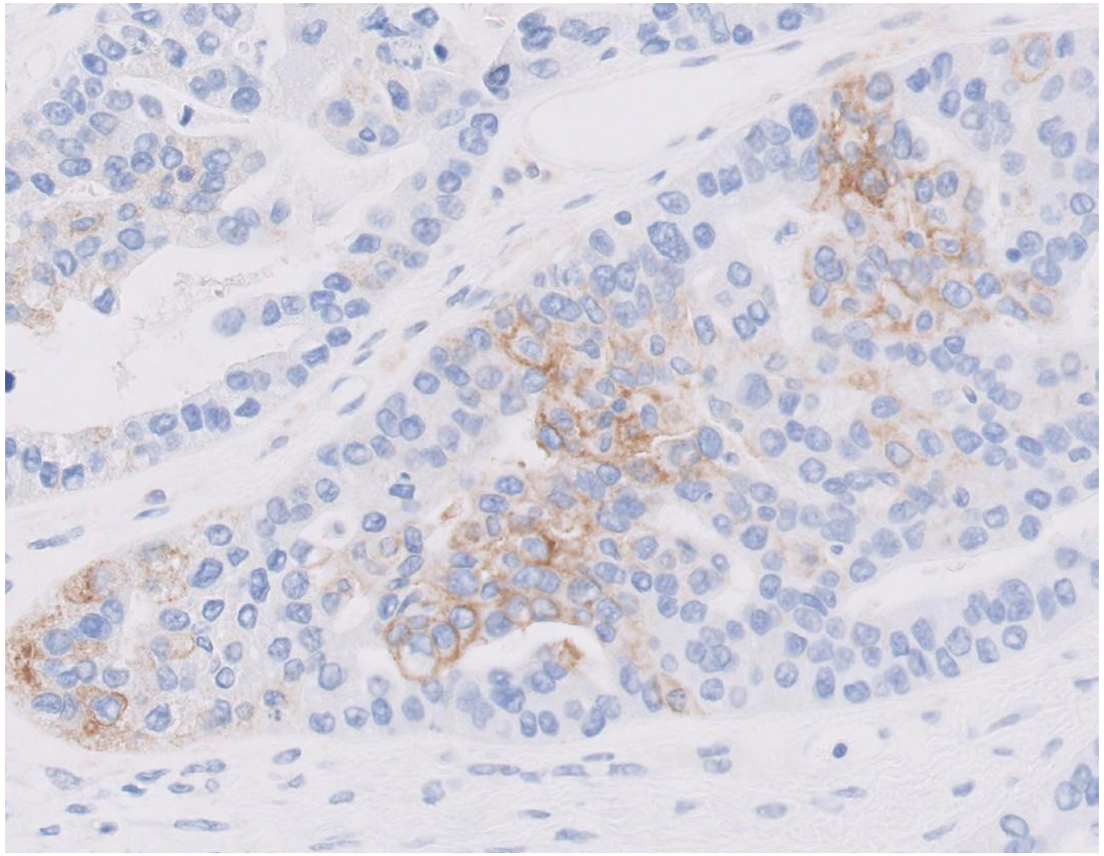
PD-L1 protein expression in EOC is determined by using Combined Positive Score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. The specimen should be considered to have PD-L1 expression if CPS ≥1.

PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying epithelial ovarian, fallopian tube, or primary peritoneal carcinoma 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.

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

PD-L1 IHC 22C3 pharmDx, Code SK006 Interpretation Manual – Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Carcinoma (EOC)



Introduction

PD-L1 IHC 22C3 pharmDx, Code SK006, is the only companion diagnostic FDA-approved as an aid in identifying patients with EOC 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 when assessing PD-L1 expression in FFPE EOC specimens. PD-L1 expression evaluation is used to identify patients for treatment with KEYTRUDA® (pembrolizumab).

The manual provides detailed scoring guidelines and some technical information from the PD-L1 IHC 22C3 pharmDx, Code SK006, Instructions for Use (IFU) to ensure high-quality staining and diagnostic assessment. Refer to the US PD-L1 IHC 22C3 pharmDx, Code SK006 IFU for full technical information and clinical data. To help familiarize you with the requirements for scoring EOC specimens stained with PD-L1 IHC 22C3 pharmDx, Code SK006, example cases of various PD-L1 expression levels are provided as references. These example cases and in-depth recommendations for interpretation of EOC specimens stained with PD-L1 IHC 22C3 pharmDx, Code SK006 can help individual laboratories achieve reproducible and reliable results.

PD-L1 IHC 22C3 pharmDx, Code SK006 is considered a qualitative immunohistochemical assay. PD-L1 expression in EOC 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.

EOC 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® (pembrolizumab). For more details on staining and interpretation, please refer to the current version of the IFU for PD-L1 IHC 22C3 pharmDx, Code SK006 on www.agilent.com.

Assay Interpretation

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

Reporting Results

To help understand what information should be reported to the treating physician, please refer to the Reporting Results section of this manual on page 25.

Photomicrographs

The included photomicrographs are of EOC unless otherwise noted.

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

Technical Considerations

Technical problems related to PD-L1 IHC 22C3 pharmDx, Code SK006 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. Confirm 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, Code SK006 Control Cell Line Slide stained with the primary antibody
- Positive and negative lab-supplied control tissues stained with the primary antibody and with the Negative Control Reagent (NCR)
- Serial section of each patient specimen stained with the NCR

Lab-supplied Control Tissue

Differences in processing and embedding in the user's laboratory may produce significant variability in results. Include positive and negative lab-supplied control tissue slides in each staining run, in addition to the PD-L1 IHC 22C3 pharmDx, Code SK006 Control Cell Line Slide.

Refer to the "Recommended Order of Slide Evaluation" table in the PD-L1 IHC 22C3 pharmDx, Code SK006 IFU for additional information on appropriate control tissue. Fix, process, and embed the control tissues in the same manner as the patient specimens. Control tissues processed differently from the patient specimens 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.

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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, 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, Code SK006 on decalcified tissues or tissues processed with other fixatives has not been validated and is not recommended.

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

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

Slide Evaluation

General Considerations

PD-L1 IHC 22C3 pharmDx, Code SK006 evaluation should be performed by a qualified pathologist using a light microscope. Details of the PD-L1 IHC 22C3 pharmDx, Code SK006 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 expression is best evaluated 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, Code SK006 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 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, Code SK006 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, Code SK006 testing.

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Evaluating Controls

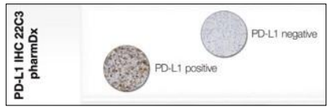


Figure 1: Each Control Cell Line Slide contains sections of cell pellets with positive and negative PD-L1 expression.

PD-L1 IHC 22C3 pharmDx, Code SK006 Control Cell Line Slide

Examine the PD-L1 IHC 22C3 pharmDx, Code SK006 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 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 ≥ 70% of cells
- ≥ 2+ average staining intensity of cells with membrane staining
- Nonspecific staining < 1+ intensity

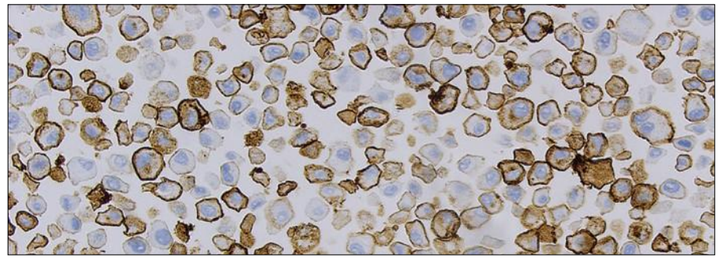


Figure 2: PD-L1 IHC 22C3 pharmDx, Code SK006 Control Cell Line Slide positive cell pellet with acceptable staining (20× magnification).

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 ≥ 1+ intensity within the boundaries of the MCF-7 cell pellet are acceptable

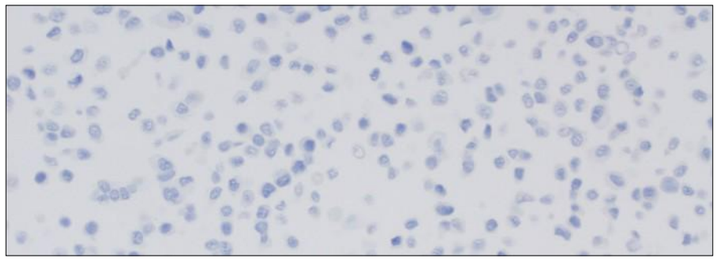


Figure 3: PD-L1 IHC 22C3 pharmDx, Code SK006 Control Cell Line Slide negative cell pellet with acceptable staining (20× magnification).

See the Control Cell Line (CCL) Appendix on page 79 for images of passing, borderline, and failing control cell line staining.

Positive Lab-supplied Control Tissue

Examine the positive control tissue slides to verify that the fixation method and epitope retrieval process are effective. The positive control tissue slides should be stained with both PD-L1 primary antibody and Negative Control Reagent. 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). 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 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 membrane staining should be observed. Nonspecific staining, including nuclear staining, should be ≤ 1+ in scorable tumor regions
- Requirements for slide stained with Negative Control Reagent: No membrane staining. Nonspecific staining, including nuclear staining, should be ≤ 1+ in scorable tumor regions

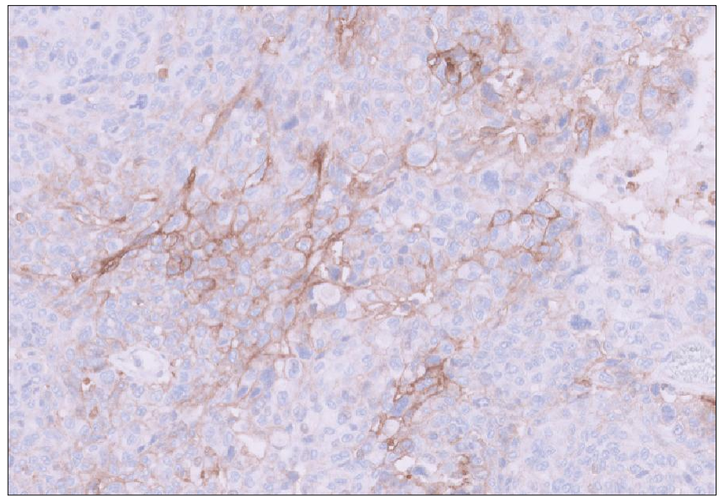


Figure 4: Ideal positive lab-supplied EOC control tissue slide stained with the PD-L1 primary antibody (20× magnification).

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 negative control tissue slides should be stained with both PD-L1 primary antibody and Negative Control Reagent. The ideal negative control tissue (slide stained with PD-L1) demonstrates no membrane staining on tumor cells (Figure 5). 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.

If staining of negative lab-supplied 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. Nonspecific staining, including nuclear staining, should be ≤ 1+ in scorable tumor regions
- Requirements for slide stained with Negative Control Reagent: No membrane staining. Nonspecific staining, including nuclear staining, should be ≤ 1+ in scorable tumor regions

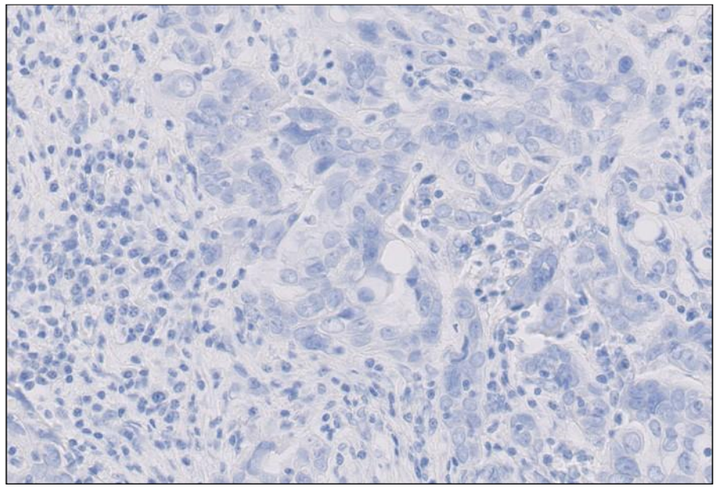


Figure 5: Ideal negative lab-supplied EOC control tissue slide stained with the PD-L1 primary antibody (20× magnification).

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.

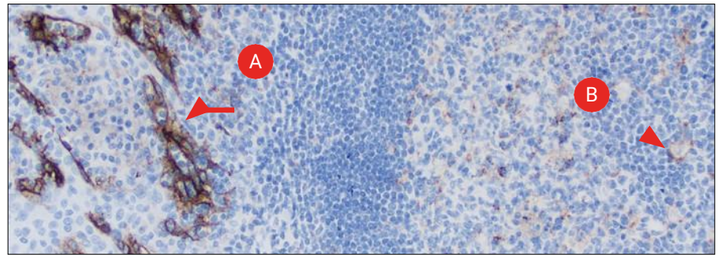


Figure 6: Tonsil tissue stained with PD-L1 primary antibody exhibiting strong membrane staining in portions of the crypt epithelium (A) and weak-to-moderate membrane staining of follicular macrophages in the germinal centers (B) (10× magnification).

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

Negative Control Reagent(NCR)

Examine the patient slide stained with the NCR to identify nonspecific staining, including nuclear staining within scorable tumor regions, that may interfere with interpreting the PD-L1 stained slide, making the specimen non-evaluable. Satisfactory performance is indicated by the absence of cell membrane staining and ≤ 1+ nonspecific staining, including nuclear staining, in scorable tumor regions (Figure 7).

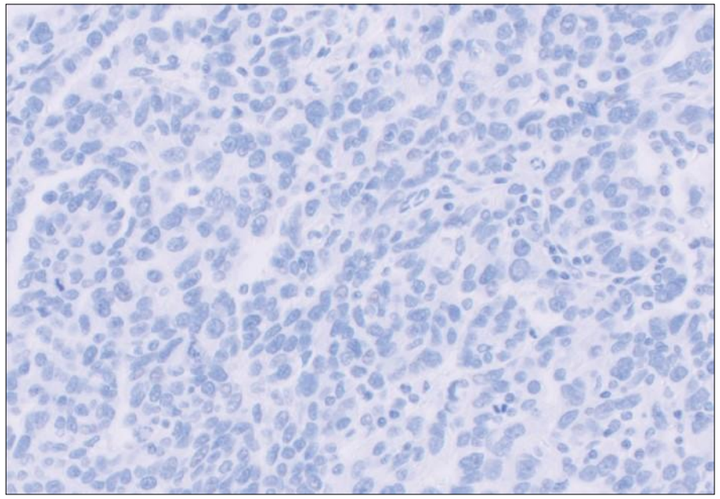


Figure 7: EOC tissue specimen slide stained with NCR (20× magnification).

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

Slide Evaluation Flowchart

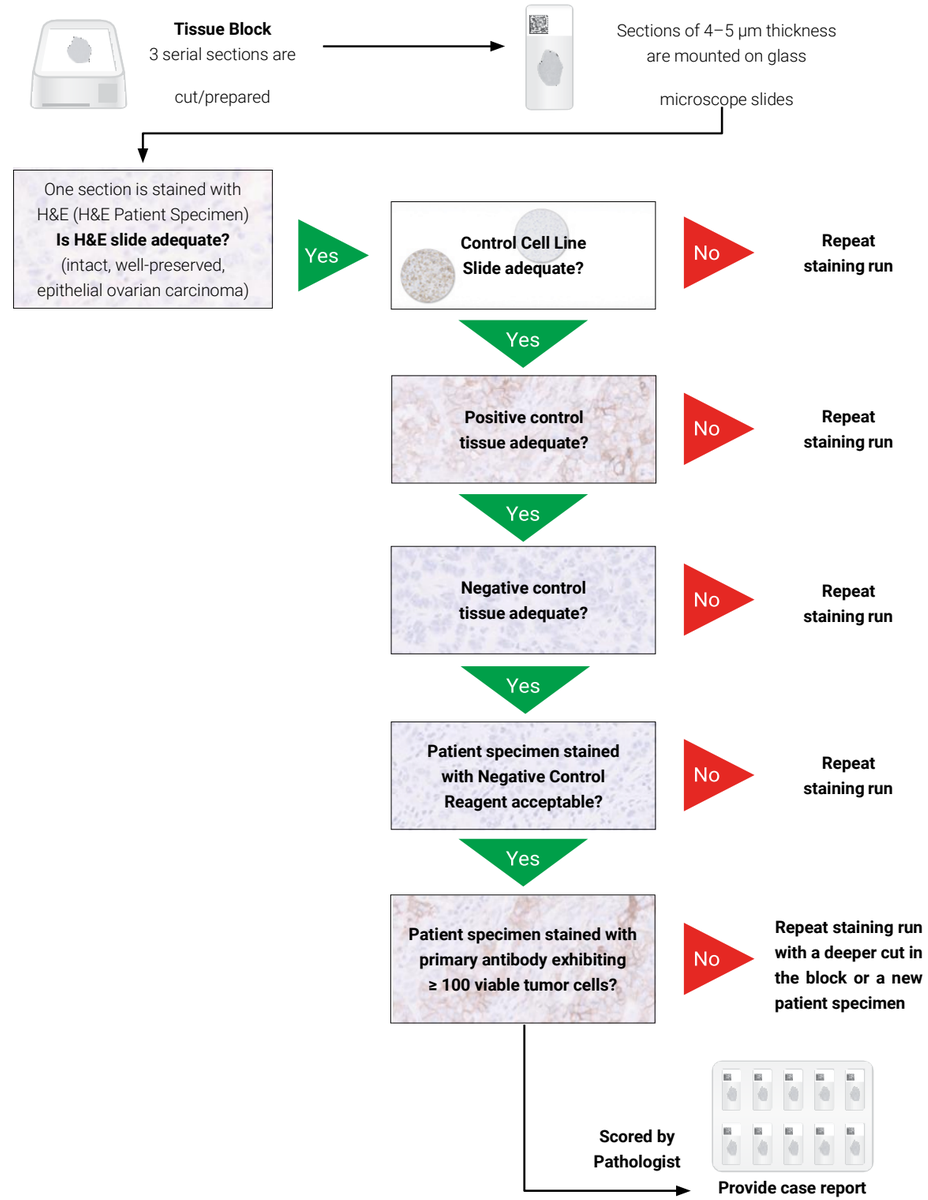


Figure 8: Recommended order of slide evaluation.

Combined Positive Score

Definition of Combined Positive Score (CPS)

PD-L1 expression in EOC 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 CPS numerator.

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 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 / 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 nonstaining (CPS denominator)
  - Determine the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) (CPS numerator; see Tables 1 and 2 on page 20 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 EOC

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	– Nonstaining tumor cells – Tumor cells with only cytoplasmic staining – Noninvasive neoplasia (Serous tubal intraepithelial carcinoma)
Immune Cells	Membrane and/or cytoplasmic* staining (at any intensity) of mononuclear inflammatory cells (MICs) within tumor nests and adjacent supporting stroma†: – Lymphocytes (including lymphocyte aggregates) – Macrophages‡ (including intraluminal and intracystic regions) Only MICs directly associated with the response to the tumor are scored.	– Nonstaining MICs – MICs (including lymphoid aggregates) not directly associated with tumor – MICs associated with noninvasive neoplasia(Serous tubal intraepithelial carcinoma) – MICs associated with benign structures – Neutrophils, eosinophils, and
Other Cells	Not included	– Benign epithelial cells – Stromal cells (fibroblasts, endothelial cells) – Necrotic cells and/or cellular debris, cystic fluid

** 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 CPS numerator.*
† 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 for EOC

Tissue Elements	Included in the Denominator	Excluded from the Denominator
Tumor Cells	All viable invasive tumor cells (PD-L1 staining or nonstaining)	– Nonviable tumor cells – Noninvasive neoplasia (Serous tubal intraepithelial carcinoma)
Immune Cells	Not included	All immune cells
Other Cells	Not included	– Benign epithelial cells – Stromal cells (fibroblasts, endothelial cells) – Necrotic cells and/or cellular debris, cystic fluid

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 Score (CPS) of various staining patterns.

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

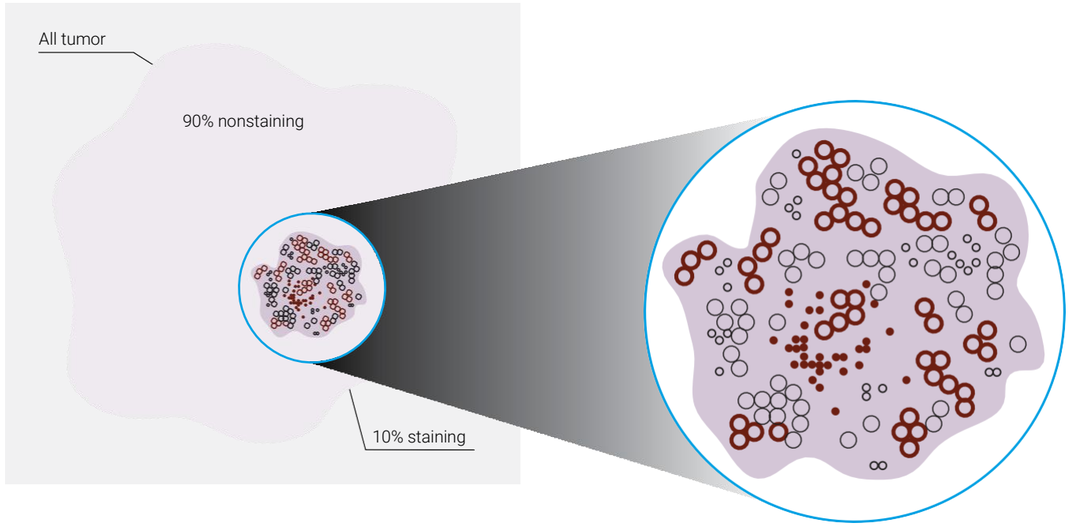
Example 1: Calculation of Combined Positive Score Based on a Small PD-L1 Staining Area

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:

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

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

Clinical Interpretation: CPS ≥ 1

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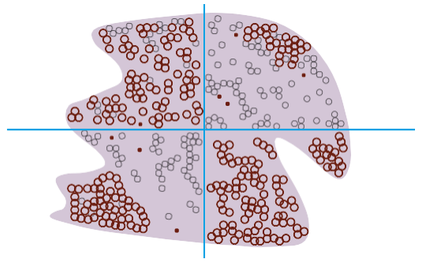
§ Including tumor cells, lymphocytes, macrophages

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

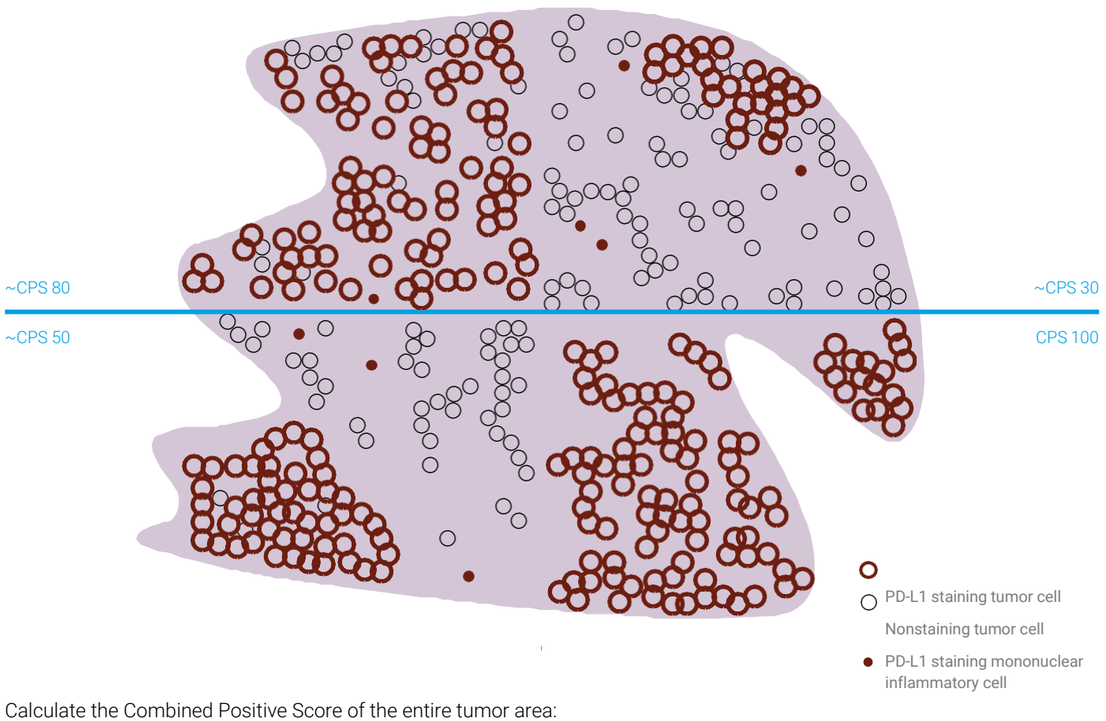
First: Visually divide the tumor area into regions with equal numbers of tumor cells.



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.

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

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:

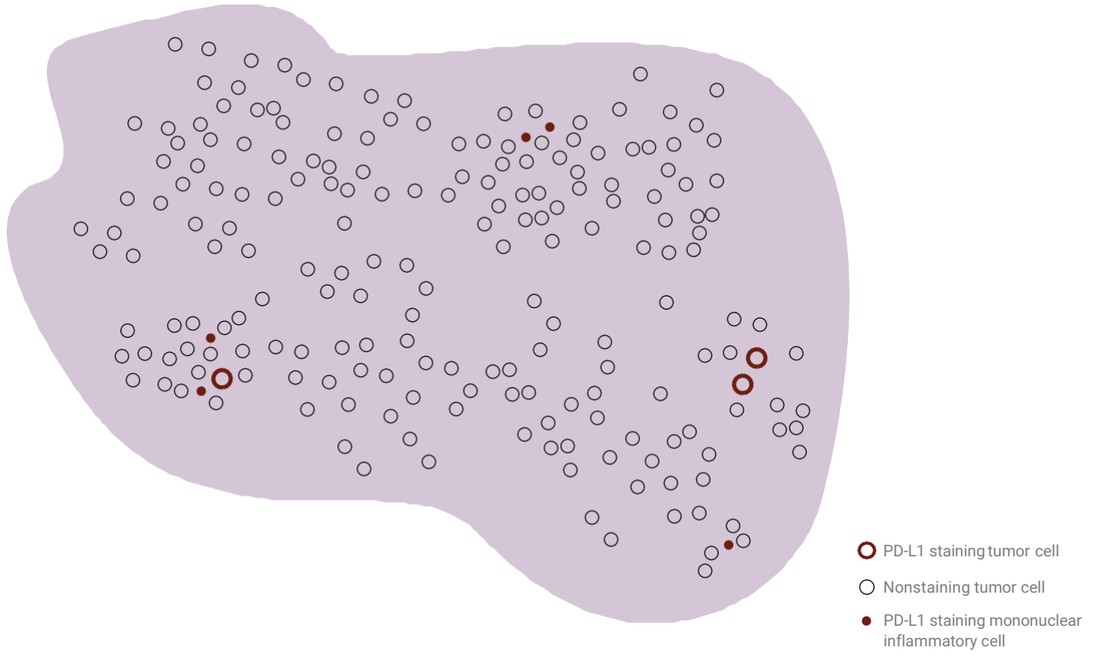
$$\frac{(80 + 30 + 50 + 100)}{4} \approx \text{CPS } 65$$

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 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: *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

Clinical Interpretation: CPS ≥ 1

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

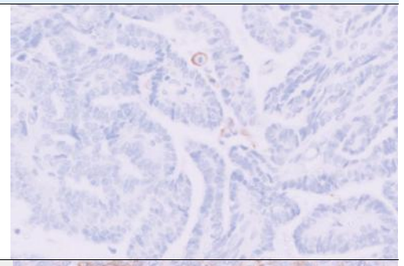
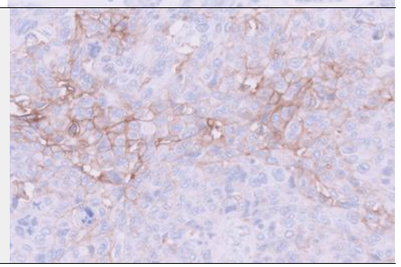
** Including tumor cells, lymphocytes, macrophages*

Figure 11: Example of a 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	

For more information, please refer to the current version of the Instructions for Use (IFU) for PD-L1 IHC 22C3 pharmDx, Code SK006.

Identifying Patients with EOC for Treatment

PD-L1 IHC 22C3 pharmDx, Code SK006 is the only companion diagnostic indicated as an aid in identifying patients with EOC for treatment with KEYTRUDA® (pembrolizumab).

Reporting Results

Suggested information to include when reporting results with PD-L1 IHC 22C3 pharmDx, Code SK006.

PD-L1 IHC 22C3 pharmDx, Code SK006 Summary of Sample Tested

Date of Run: _____

PD-L1 IHC 22C3 pharmDx, Code SK006 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, Code SK006 Result to Treating Physician

Combined Positive Score: _____

CPS < 1: ☐ CPS ≥ 1: ☐

Comments to Treating Physician:

– See the KEYTRUDA® (pembrolizumab) prescribing information for details.

Combined Positive Score Summary and Examples

Key Considerations in Scoring PD-L1 IHC 22C3 pharmDx, Code SK006 Stained Specimens

By definition, PD-L1 staining cells in EOC are:

- Viable tumor cells with perceptible and convincing partial or complete linear membrane staining (at any intensity) that is perceived as distinct from cytoplasmic staining
- Lymphocytes and macrophages (mononuclear inflammatory cells, MICs) within 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 EOC 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 EOC unless otherwise noted in the figure caption.

Image Guide for Interpretation of PD-L1 IHC 22C3 pharmDx, Code SK006 Staining in EOC

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 20 for additional CPS inclusion/exclusion criteria). Below is an image guide to aid in accurately determining CPS in EOC specimens. All images are EOC unless otherwise noted in the figure caption.

Tumor Cells

Tumor Cell Size

EOC 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 CPS denominator. Well- differentiated carcinoma may exhibit larger tumor cells 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 CPS denominator, the greater the number of PD-L1 staining tumor cells, lymphocytes, and macrophages that are needed in the CPS 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 Tumor Cell Size

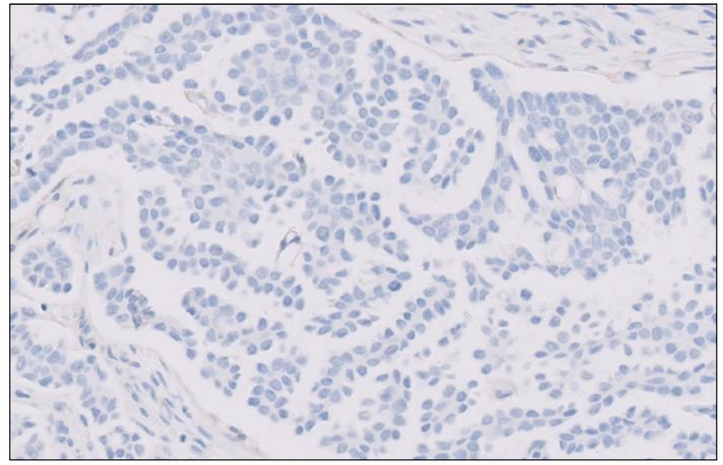


Figure 12a: EOC specimen with small sized tumor cells (20× magnification).

Medium Tumor Cell Size

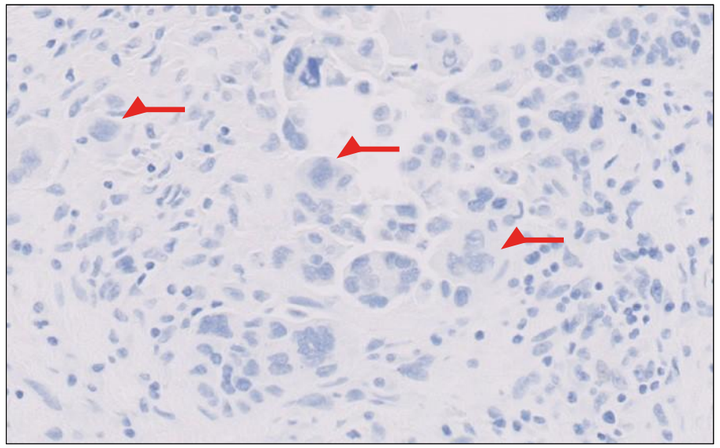


Figure 12b: EOC specimen with medium sized tumor cells (arrows) (20× magnification).

Large Tumor Cell Size

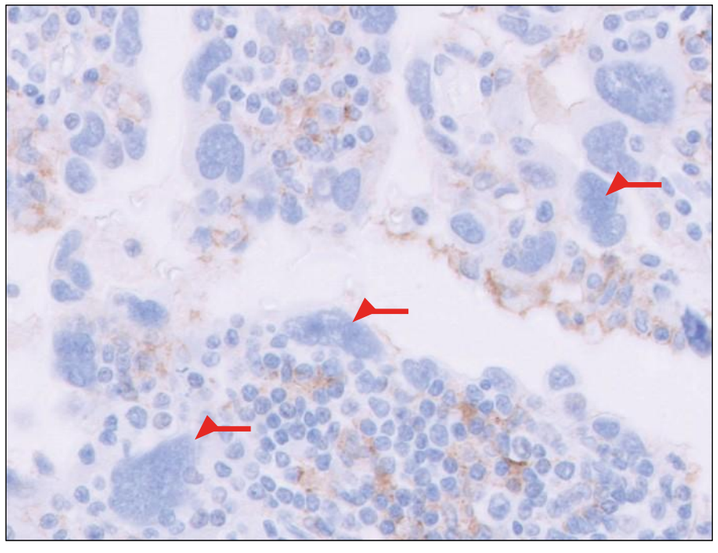


Figure 12c: EOC specimen with large sized tumor cells (20× magnification).

Key Point

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

Tumor and Immune Cells That Are Difficult to Distinguish

Tumor cells and immune cells may be difficult to distinguish from each other when examining the slide stained with the PD-L1 primary antibody 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 CPS denominator.

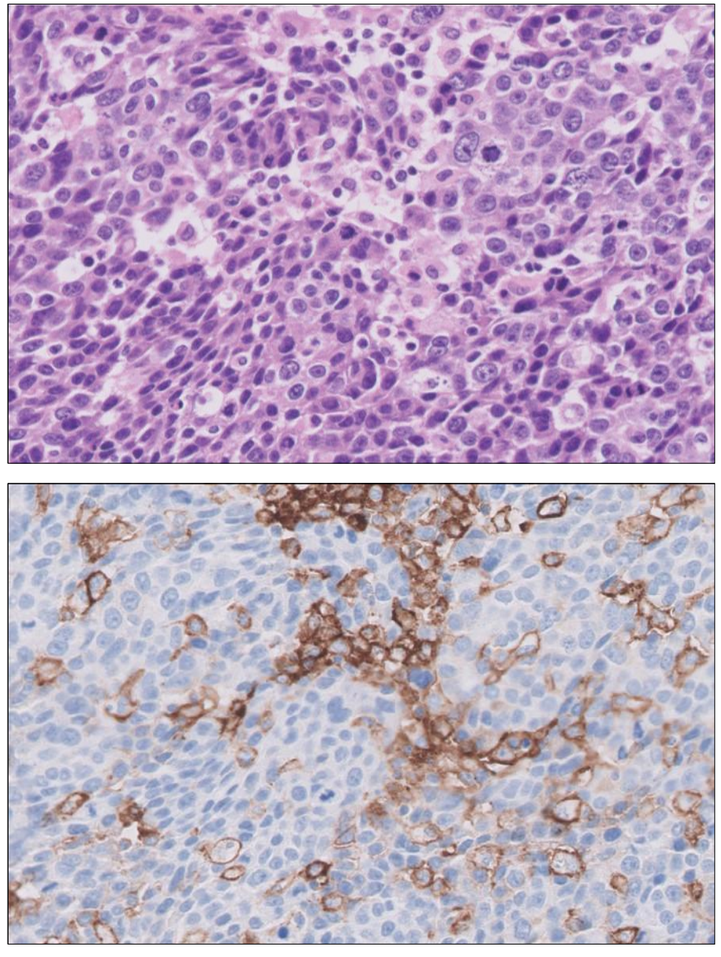


Figure 13a: EOC specimen exhibiting an area of admixed tumor and immune cells that are difficult to distinguish from each other – H&E (top) and corresponding PD-L1 field (20× magnification).

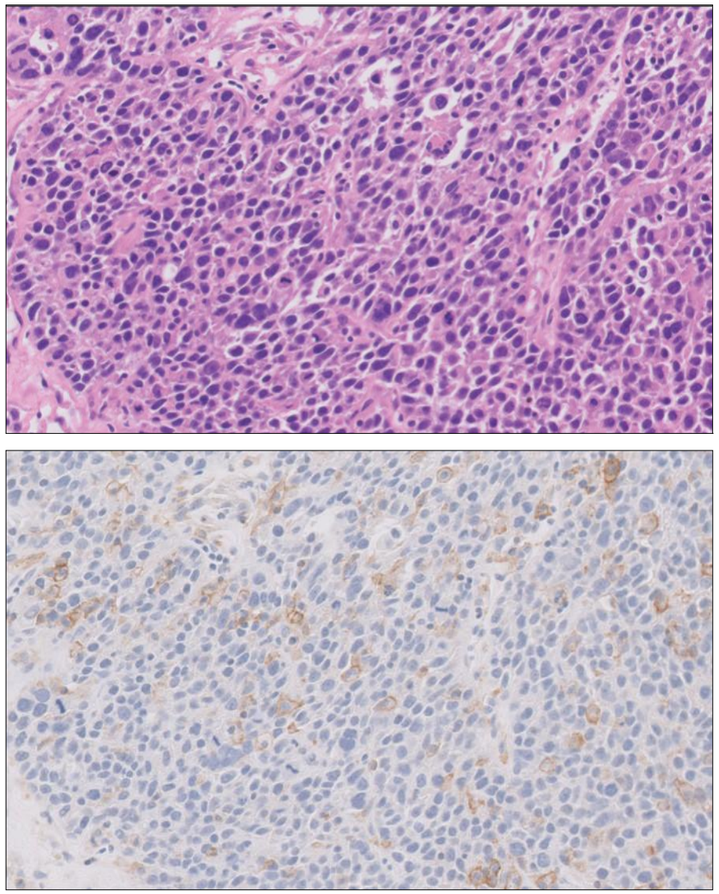


Figure 13b: EOC specimen exhibiting an area of admixed tumor and immune cells that are difficult to distinguish from each other – H&E (top) and corresponding PD-L1 field (20× magnification).

Key Point

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

Linear Membrane Staining

Tumor cells exhibiting perceptible and convincing partial 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.

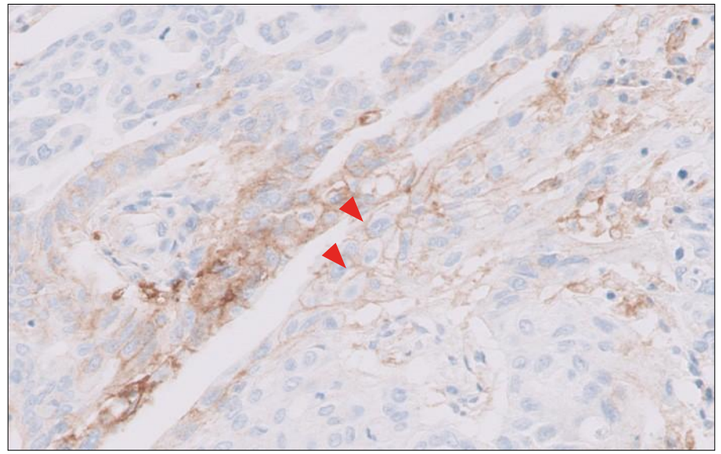


Figure 14a: EOC specimen stained with PD-L1 primary antibody exhibiting 1+ linear membrane staining of tumor cells (arrows) (20× magnification).

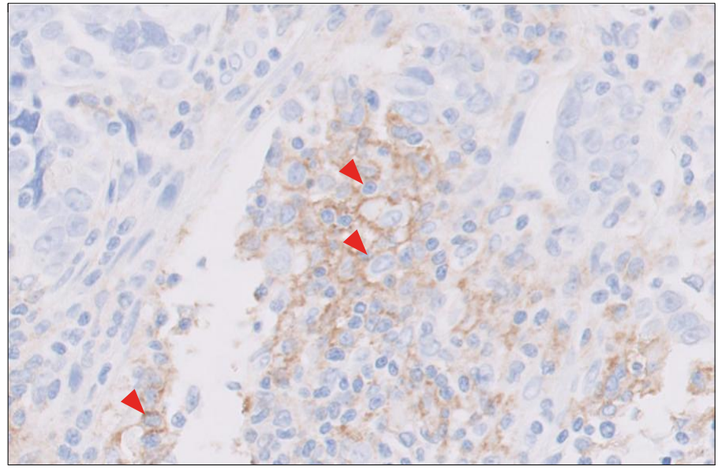


Figure 14b: EOC specimen stained with PD-L1 primary antibody exhibiting 2+ linear membrane staining of tumor cells (arrows) (20× magnification).

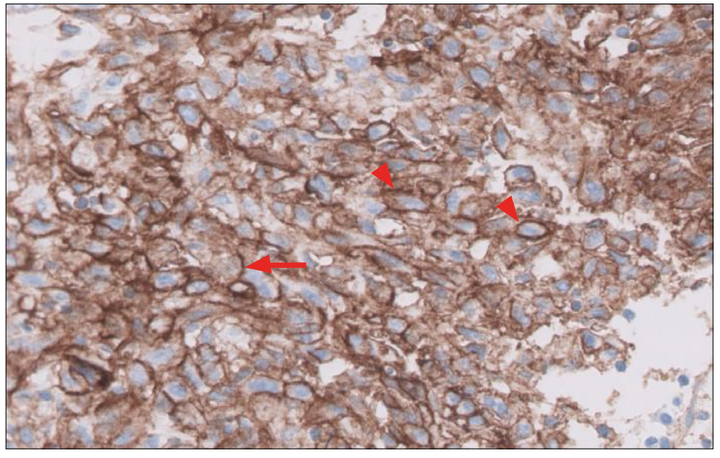


Figure 14c: EOC specimen stained with PD-L1 primary antibody exhibiting 3+ linear membrane staining of tumor cells (20× magnification).

Key point

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

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.

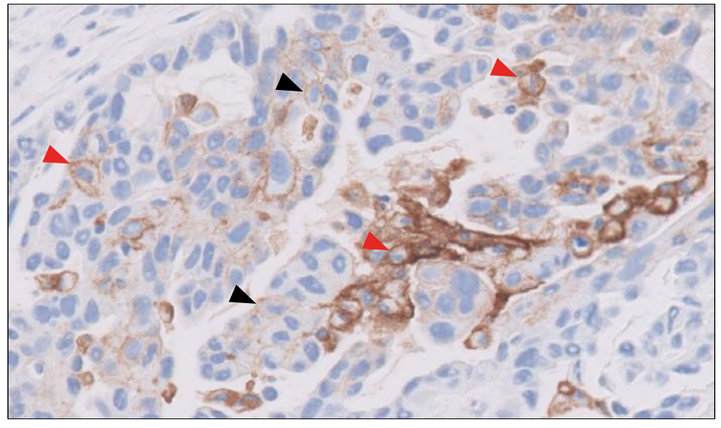


Figure 15: EOC specimen stained with PD-L1 primary antibody exhibiting partial (black arrows) and complete (red arrows) linear membrane staining of tumor cells (20× magnification).

Key point

Perceptible and convincing partial or complete 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 (≥ 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. If linear membrane staining is distinct from cytoplasmic staining, then the cell should be included in the CPS numerator.

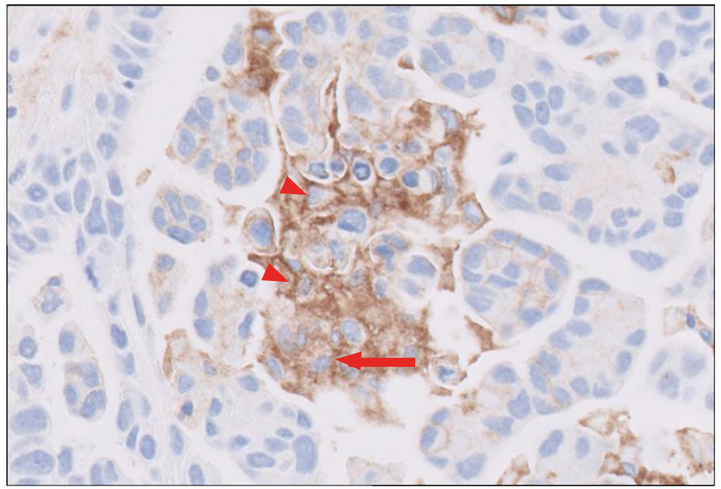


Figure 16: EOC specimen stained with PD-L1 primary antibody exhibiting tumor cells with perceptible and convincing linear membrane staining distinct from cytoplasmic staining (arrows) (20× magnification).

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

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.

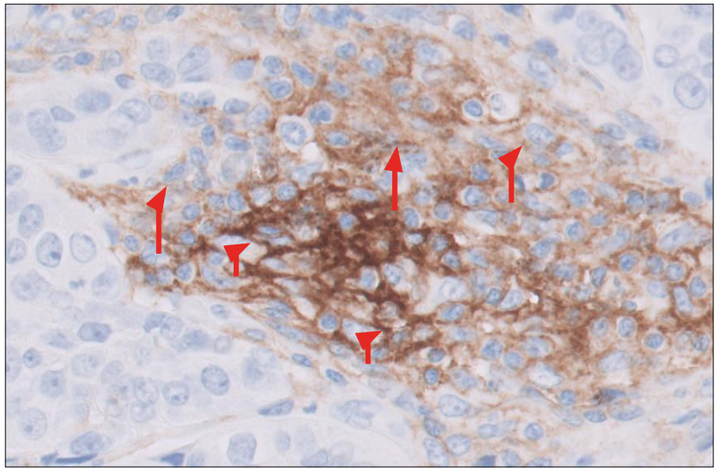


Figure 17: EOC specimen stained with PD-L1 primary antibody exhibiting granular membrane staining of tumor cells (arrows) (20× magnification).

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

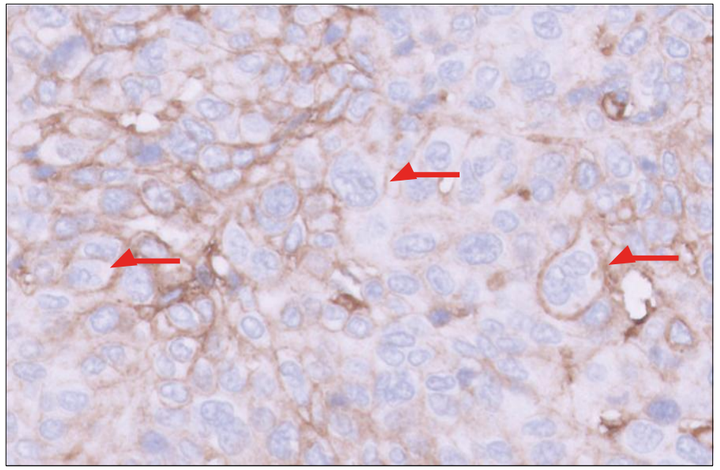


Figure 18: EOC specimen stained with PD-L1 primary antibody exhibiting PD-L1 staining multinucleate tumor cells (arrows) (20× magnification).

Key Point

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

PD-L1 IHC 22C3 pharmDx, Code SK006 Interpretation Manual – Ovarian Carcinoma

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 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.

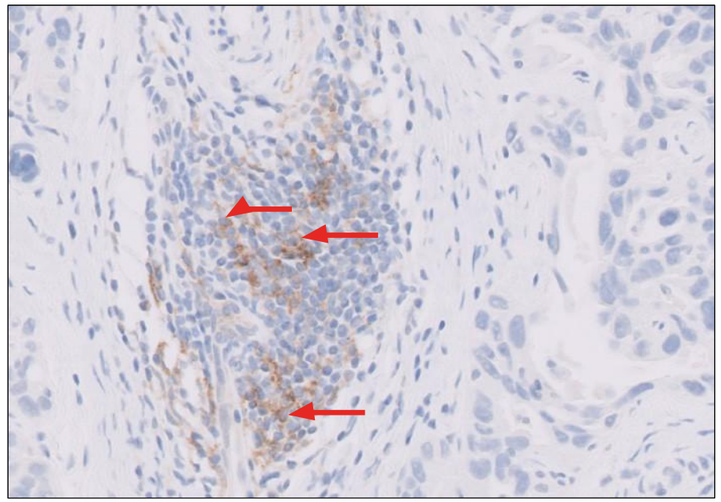


Figure 19: EOC specimen stained with PD-L1 primary antibody exhibiting PD-L1 staining tumor-associated lymphocytes (arrows) (20× magnification).

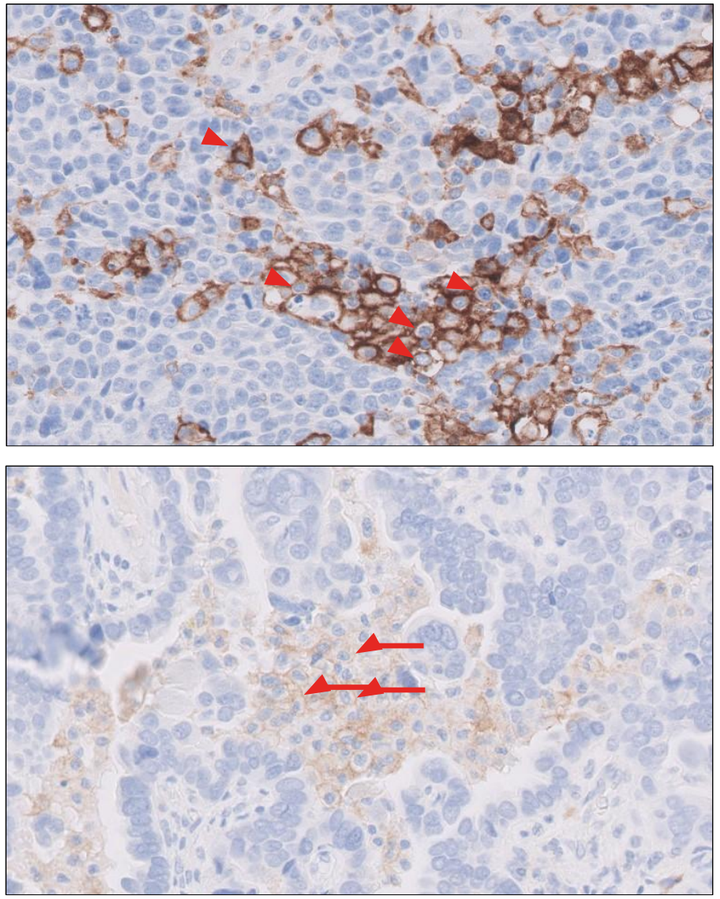


Figure 20a: EOC specimen stained with PD-L1 primary antibody exhibiting PD-L1 staining tumor-associated macrophages (arrows) (20× magnification).

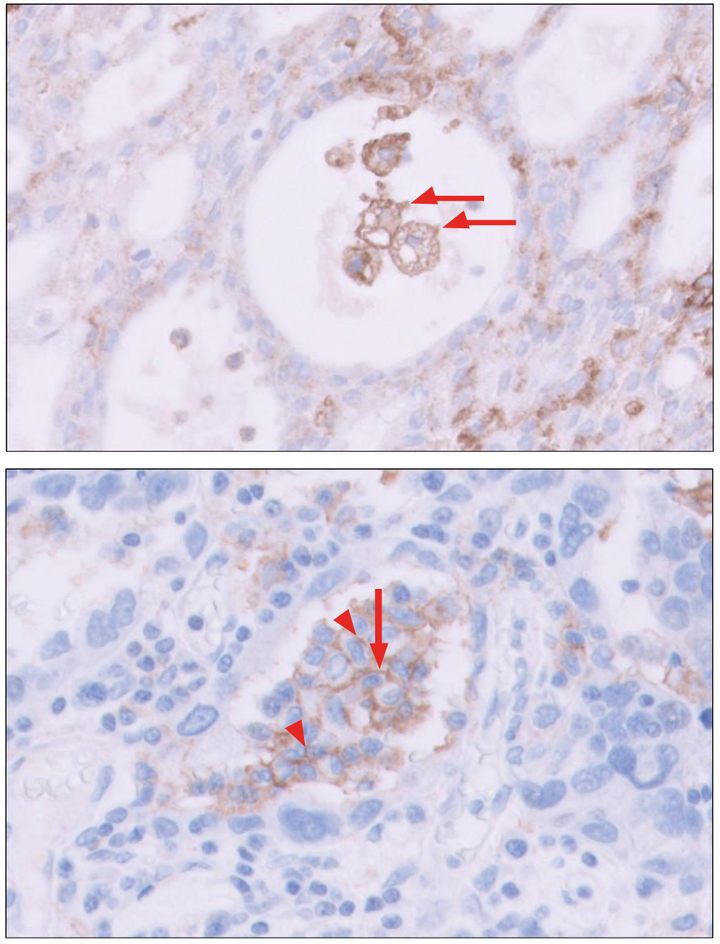


Figure 20b: EOC specimen stained with PD-L1 primary antibody exhibiting PD-L1 staining intraluminal/intracystic macrophages within tumor that should be included in the CPS numerator (arrows) (20× magnification).

Multinucleated Giant Cells

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

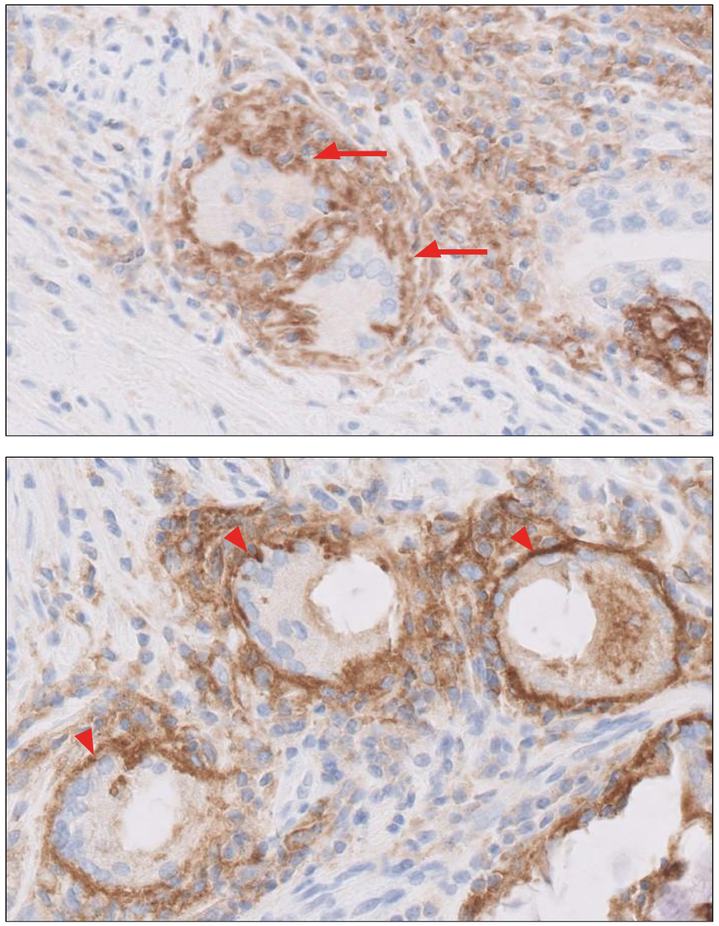


Figure 21: EOC specimen stained with PD-L1 primary antibody exhibiting PD-L1 staining tumor-associated multinucleated giant cells (arrows) (20× magnification).

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 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 small tumor nest is in the approximate center of a 20× field or such that the large tumor mass fills approximately half of a 20× field. MICs that lie within this field should be included in scoring. MICs outside of this field should be excluded from scoring as long as they do not surround neighboring tumor. See Figures 22a–22c for an example of determining which PD-L1 staining MICs are included in the CPS numerator.

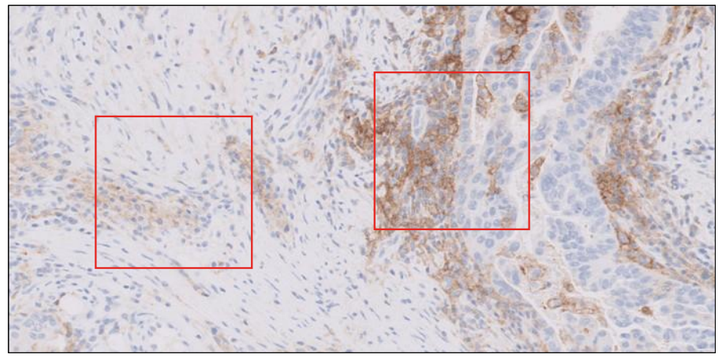


Figure 22a: At 10× magnification, two areas of PD-L1 staining MICs are visible. Following the instructions above, zoom in to 20× magnification on each field to determine which PD-L1 staining MICs to include in the CPS numerator (10× magnification).

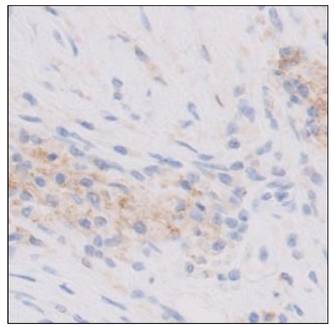


Figure 22b: Tumor cells are absent from this 20x field containing PD-L1 staining MICs, thus none of these MICs should be included in the CPS numerator (20× magnification).

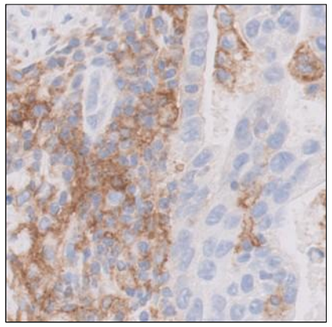


Figure 22c: When positioning the tumor such that it fills approximately half of a 20× field, PD-L1 staining MICs that are present within the same field should be included in the CPS numerator as long as they are directly related to the response against the tumor (20× magnification).

Cells Excluded from CPS

Only tumor cells exhibiting PD-L1 membrane staining and tumor-associated MICs exhibiting PD-L1 membrane and/or cytoplasmic staining should be included in the CPS numerator. On the following pages 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 Tables 1 and 2 on 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.

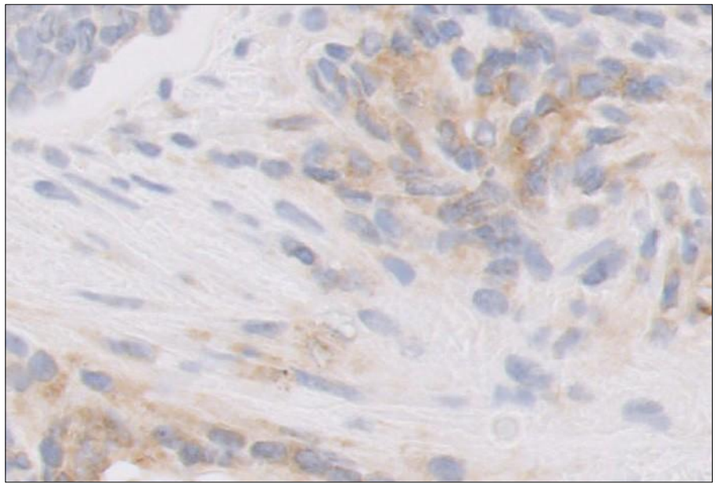


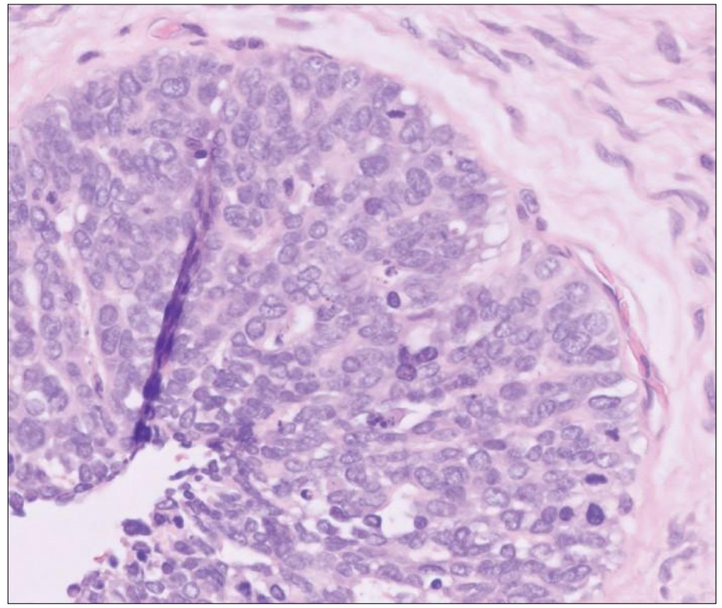
Figure 23: EOC specimen stained with PD-L1 primary antibody exhibiting only cytoplasmic staining of tumor cells (20× magnification).

Key point

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

Noninvasive neoplasia (Serous tubal intraepithelial carcinoma)

Both PD-L1 staining and nonstaining noninvasive neoplasia as well as PD-L1 staining MICs associated with noninvasive neoplasia should be excluded from the CPS calculation.



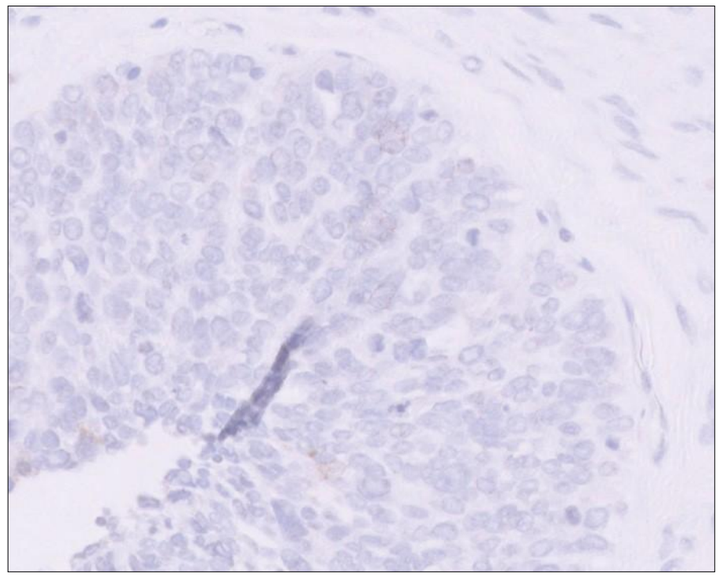


Figure 24: Serous tubal intraepithelial carcinoma and PD-L1 staining MICs associated with Serous tubal intraepithelial carcinoma as seen in the surface epithelium of fallopian tube should be excluded from the CPS calculation – H&E (top) and corresponding PD-L1 field (bottom)

(20× magnification).

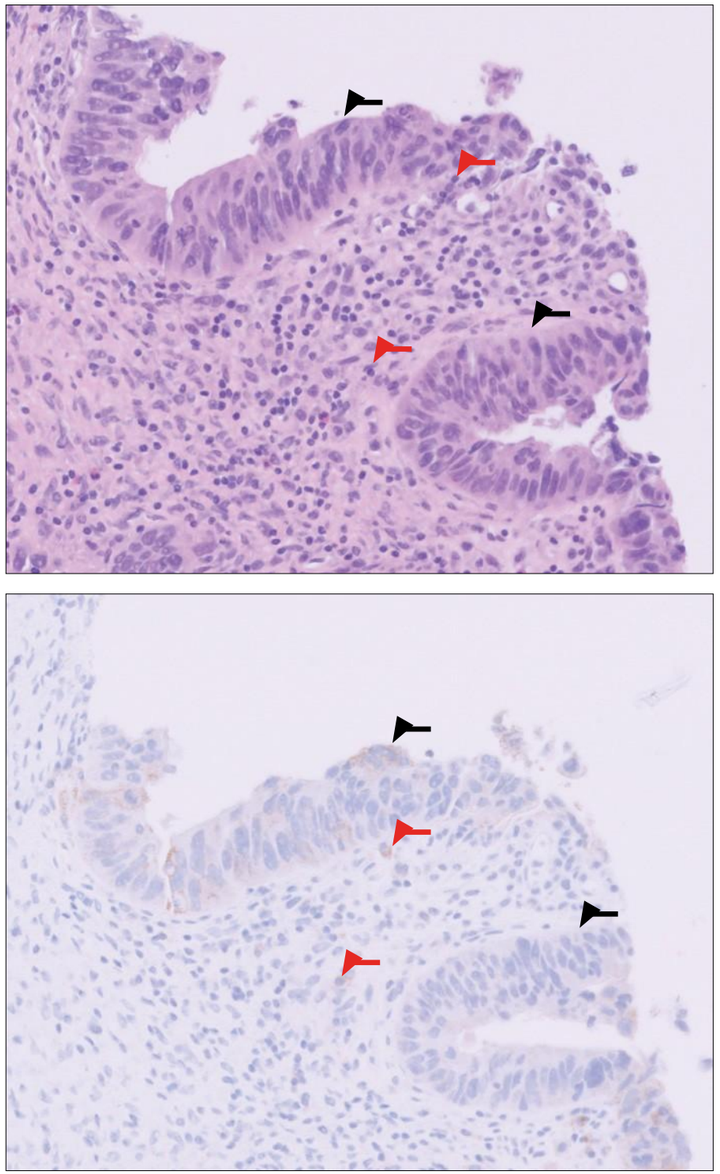


Figure 25: Serous tubal intraepithelial carcinoma (black arrows) and MICs associated with Serous tubal intraepithelial carcinoma as seen in the surface epithelium of fallopian tube (red arrows) can exhibit PD-L1 staining but should be excluded from the CPS calculation – H&E (top) and corresponding PD-L1 field (bottom) (20× magnification).

Key point

Noninvasive neoplasia (Serous tubal intraepithelial carcinoma)and MICs associated with noninvasive neoplasia can exhibit PD-L1 staining and should be excluded from the CPS calculation

Other Immune Cells Excluded from CPS

Various types of immune cells can exhibit PD-L1 staining, but only PD-L1 staining tumor-associated lymphocytes and macrophages should be included in the CPS numerator. Refer to page 41 for the immune cell inclusion/exclusion 20× rule. PD-L1 staining MICs can sometimes be associated with necrosis or benign structures and these non-tumor associated PD-L1 staining MICs should be excluded from the CPS numerator. Neutrophils, eosinophils, and plasma cells should be excluded from the CPS calculation.

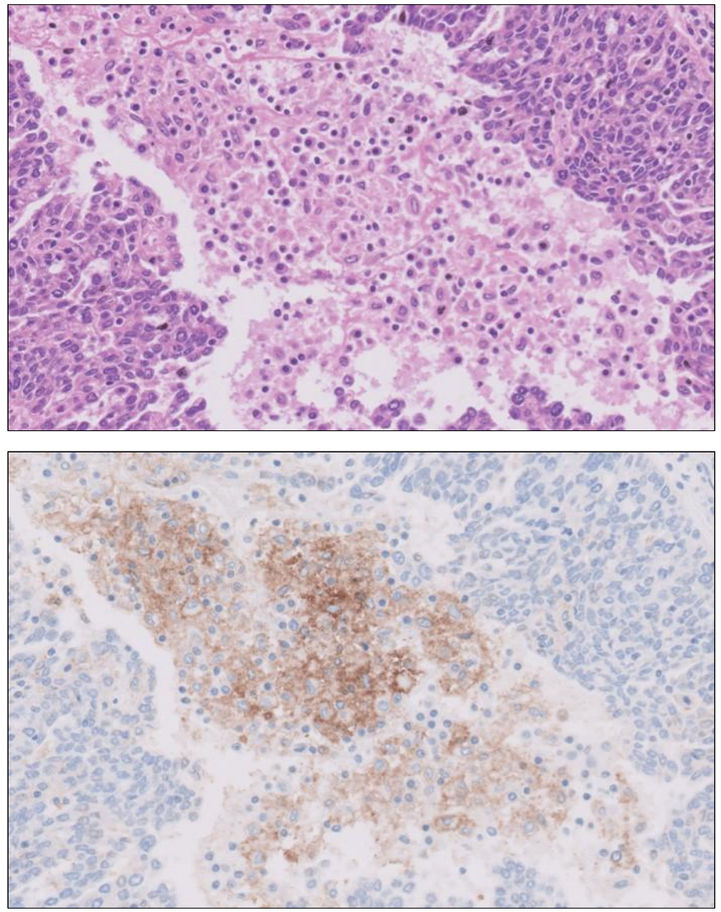


Figure 26: PD-L1 staining MICs that are adjacent to tumor but associated with necrosis are not considered tumor-associated and should be excluded from the CPS numerator – H&E (top) and corresponding PD-L1 field (bottom) (20× magnification).

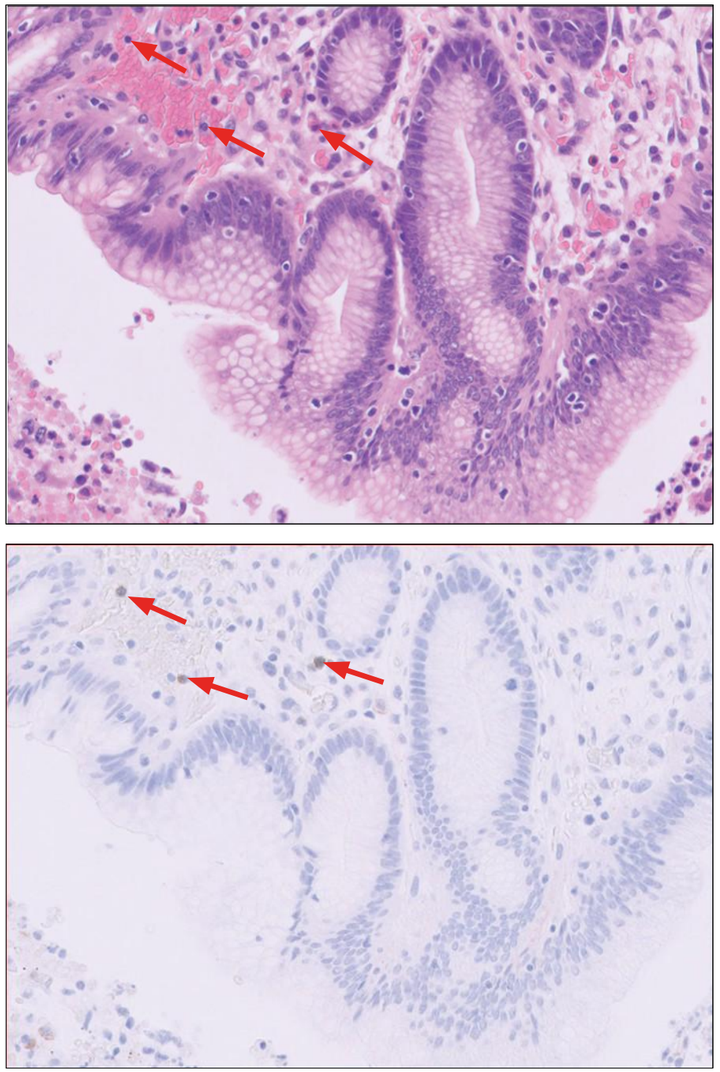


Figure 27: PD-L1 staining MICs associated with benign epithelium (arrows) should be excluded from the CPS calculation – H&E (top) and corresponding PD-L1 field (bottom) (20× magnification). Note: gastric or GEJ adenocarcinoma depicted.

Key point

Non-tumor associated PD-L1 staining MICs such as PD-L1 staining MICs associated with necrosis and benign structures should be excluded from the CPS numerator

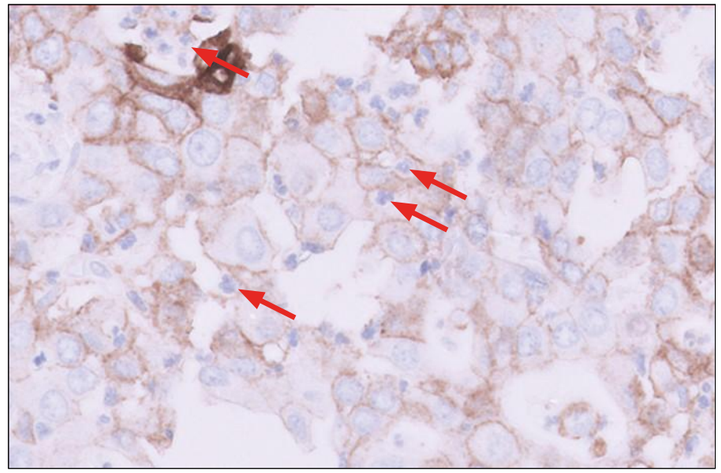


Figure 28: Gastric or gastroesophageal junction (GEJ) adenocarcinoma specimen stained with PD-L1 primary antibody exhibiting PD-L1 staining neutrophils (arrows) (20× magnification).

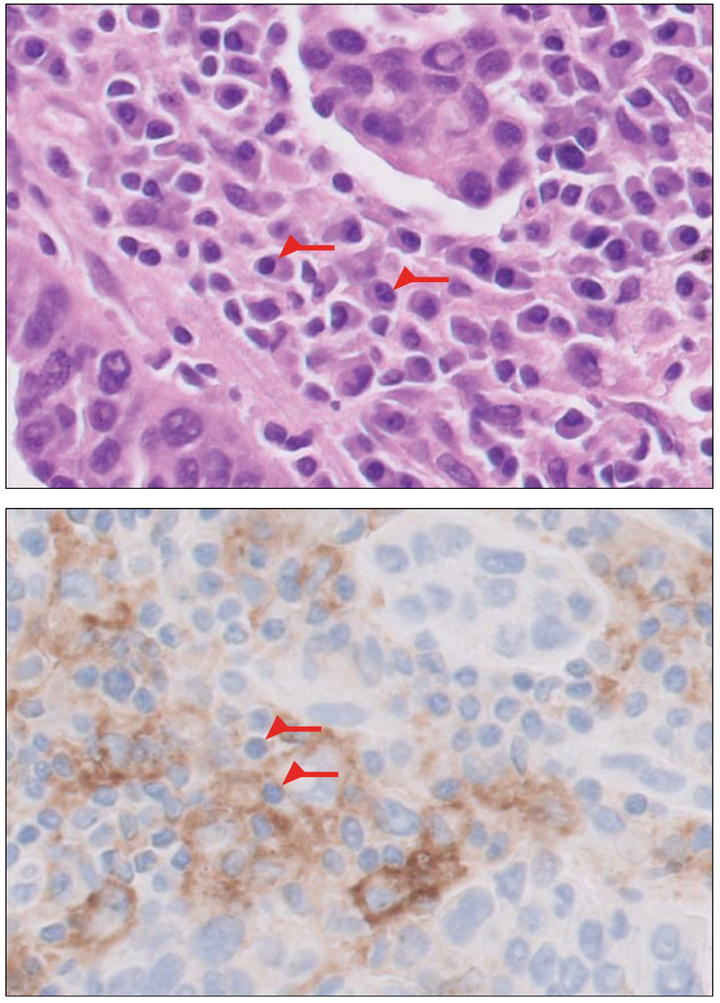


Figure 29: EOC specimen with focus containing PD-L1 staining plasma cells (red arrows) which should be excluded from the CPS calculation – H&E (top) and corresponding PD-L1 field (bottom) (20× magnification).

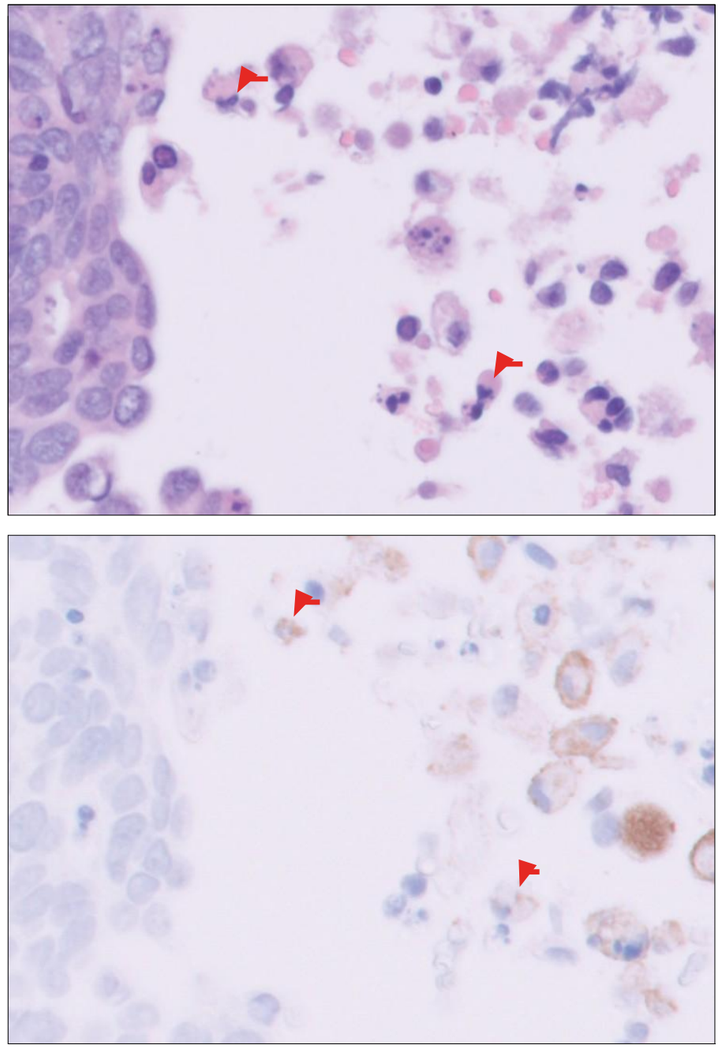


Figure 30: EOC specimen with focus containing PD-L1 staining eosinophils (arrows) which should be excluded from the CPS calculation – H&E (top) and corresponding PD-L1 field (bottom) (20× magnification).

Key point

Neutrophils, eosinophils, and plasma cells should be excluded from the CPS calculation

Other Cells Excluded from CPS

Benign Epithelium

Benign epithelium can exhibit PD-L1 staining, but should be excluded from the CPS calculation.

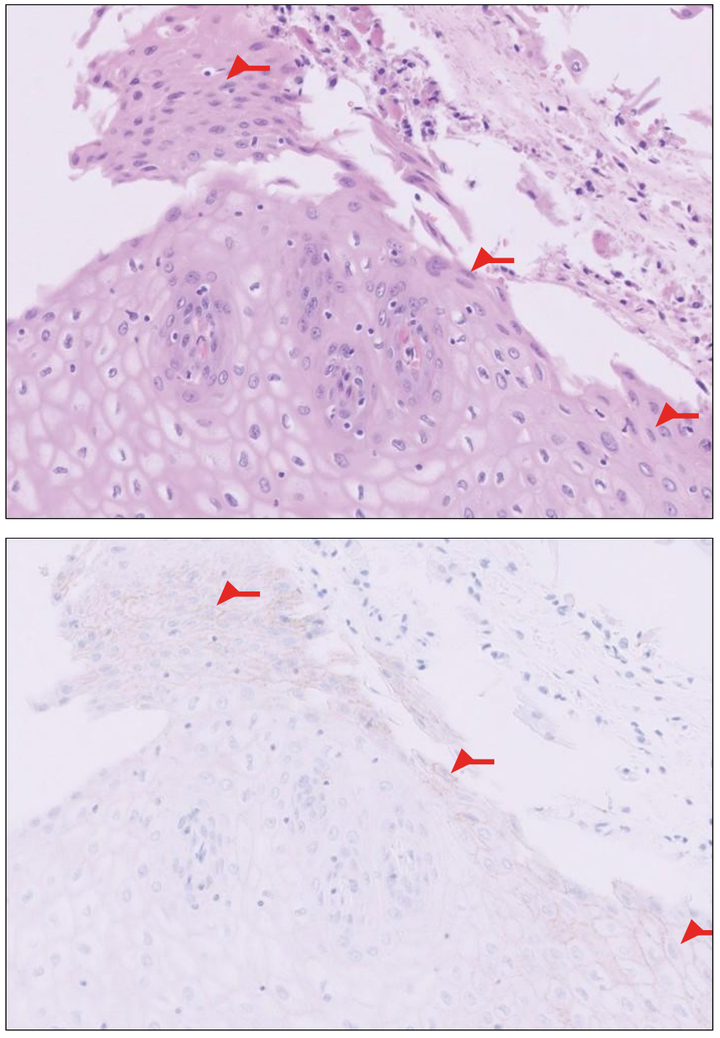


Figure 31: Tissue specimen exhibiting staining of benign epithelium (arrows) that should be excluded from the CPS calculation – H&E (top) and corresponding PD-L1 field (bottom) (20× magnification). Note: gastric or GEJ adenocarcinoma depicted.

Stromal Cells (Fibroblasts and Endothelial Cells)

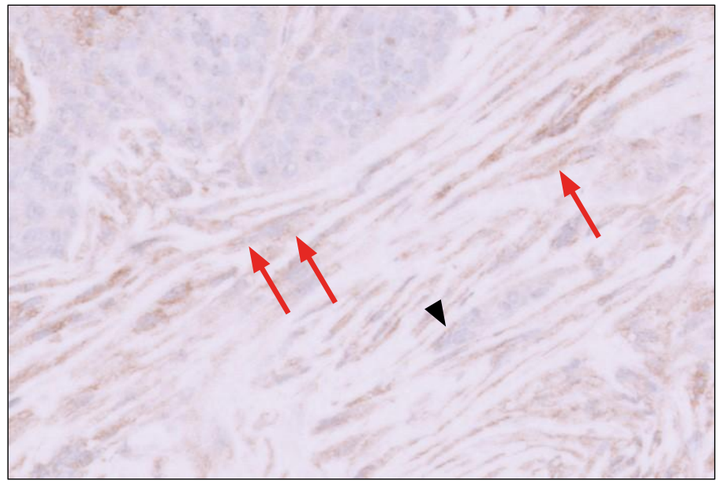


Figure 32a: EOC specimen stained with PD-L1 primary antibody exhibiting PD-L1 staining fibroblasts (red arrows) and endothelial cells (black arrow) (20× magnification).

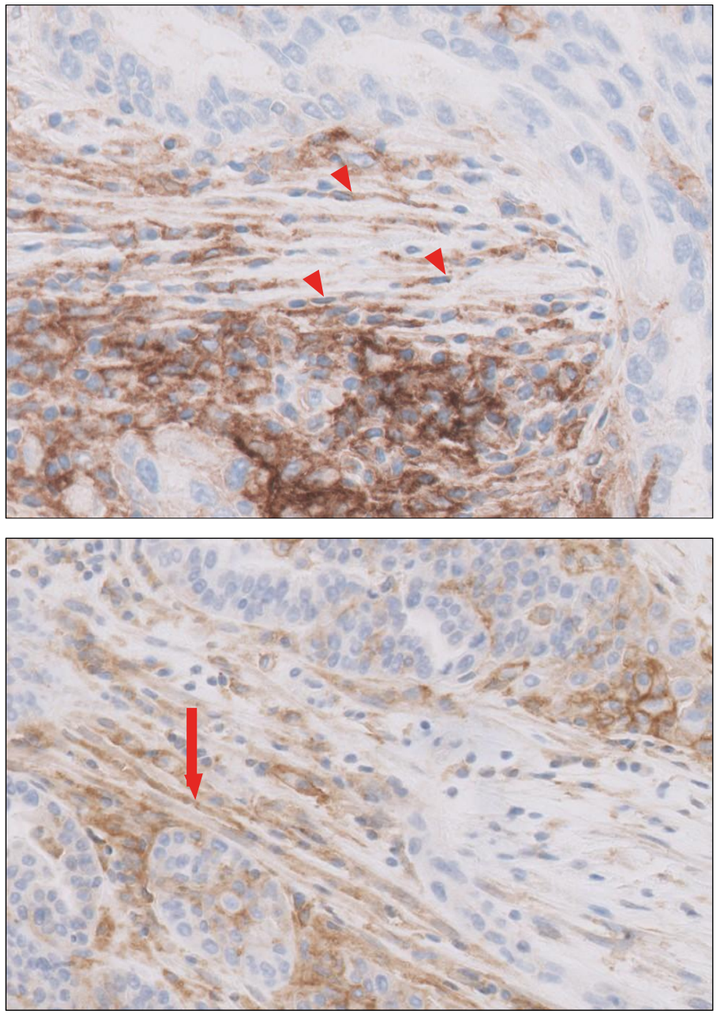


Figure 32b: EOC specimen stained with PD-L1 primary antibody exhibiting PD-L1 staining stromal cells (arrows) (20× magnification).

Key point

Benign epithelium and stromal cells (fibroblasts, endothelial cells) may exhibit PD-L1 staining but should be excluded from the CPS calculation

Artifacts

The following pages provide examples of artifacts you may see in PD-L1 IHC 22C3 pharmDx, Code SK006 stained specimens. All artifacts depicted in this section can be seen in different tumor types, including EOC. The tumor type depicted in each image is specified in the figure caption.

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.

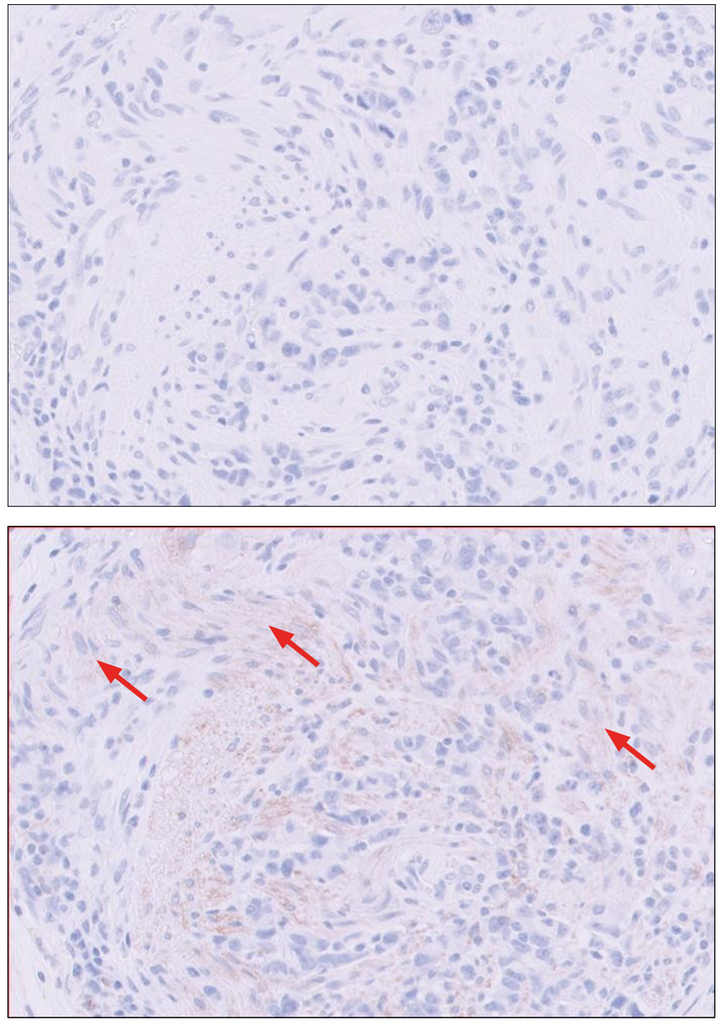


Figure 33a: Gastric or GEJ adenocarcinoma specimen exhibiting ≤ 1+ intensity specific nonscorable cytoplasmic staining of peripheral nerve cells (arrows). Absence of similar staining in the NCR-stained slide (top) indicates that this cytoplasmic staining in the PD-L1 slide (bottom) is specific, but benign cells such as peripheral nerve cells should be excluded from scoring (20× magnification).

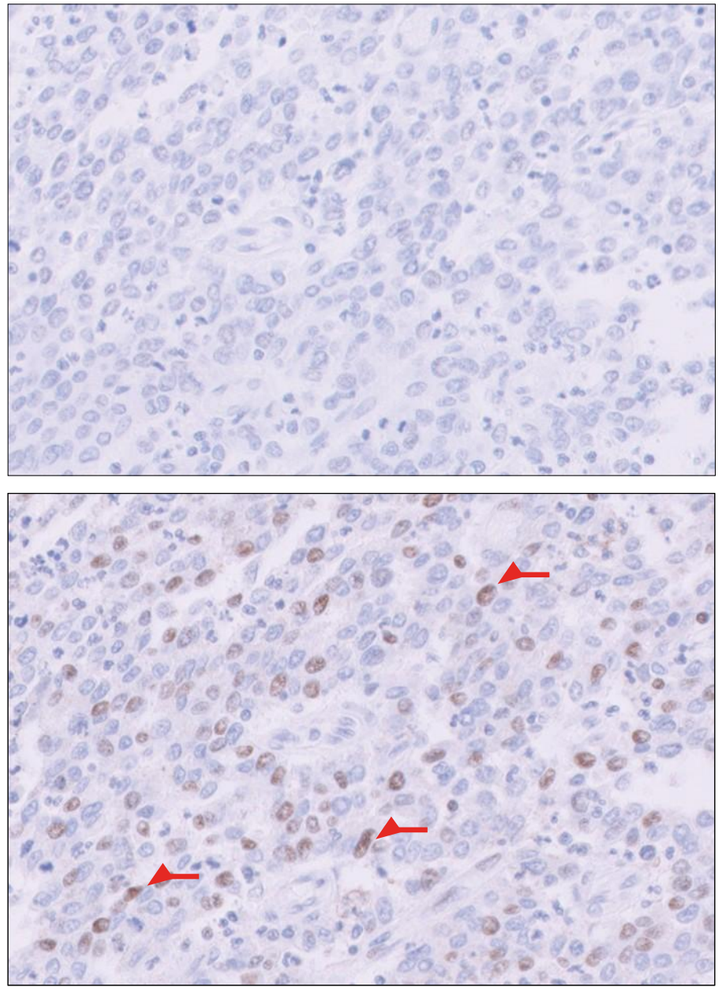


Figure 33b: Gastric or GEJ adenocarcinoma specimen with nuclear staining of tumor cells absent from the NCR-stained slide (top) and present in the PD-L1 stained slide (bottom) (arrows), indicating that this nuclear staining is specific staining. Since the nucleus is a cell compartment that is not scorable, and the nuclear reactivity does not interfere with evaluation of membrane staining on tumor cells, the PD-L1 stained slide should be considered evaluable (20× magnification).

Nonspecific Staining

Nonspecific staining, including nonspecific nuclear 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 and prior to initiating run. Ensure that slide drying does not occur during the staining procedure
- Cross-reactivity of the secondary antibody in the detection system
- Reagent trapping (tissue folding; tissue drying; hydrophobic or ionic interactions with "sticky" tissues or substances such as cartilage, muscle fibers, dense fibrosis, mucin, necrotic debris)

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

All specimens must have ≤ 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 IHC 22C3 pharmDx, Code SK006 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 ≤ 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, Code SK006 is rare.

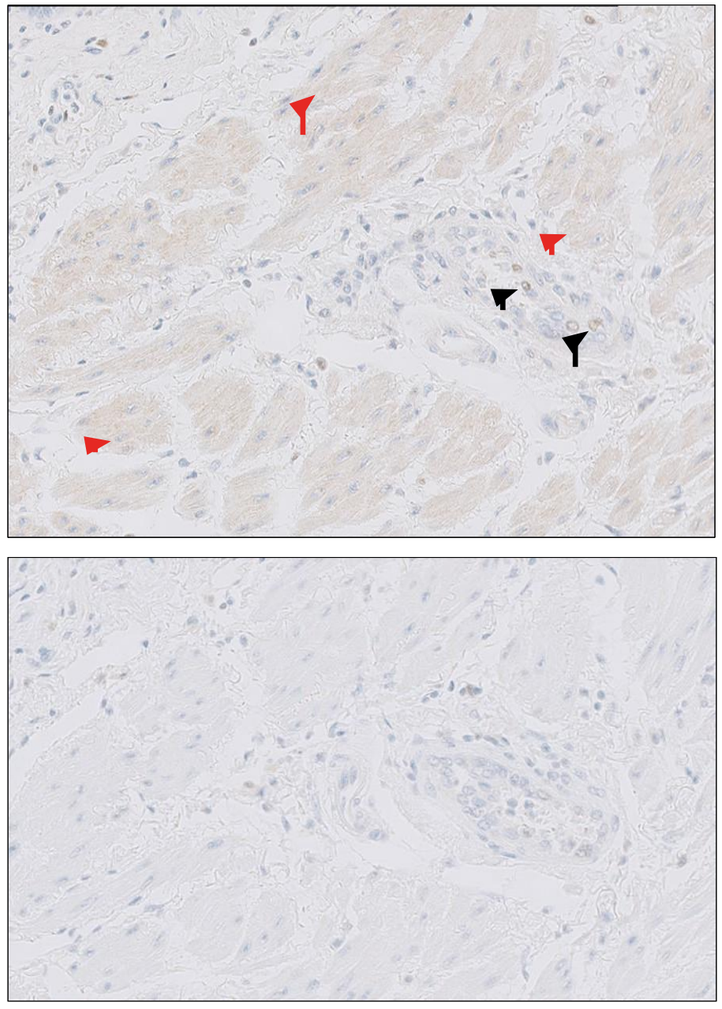


Figure 34a: Esophageal adenocarcinoma specimen stained with NCR (top) exhibiting acceptable (≤ 1+ intensity) nonspecific staining on smooth muscle cells (red arrows) and occasional endothelial cell nuclei (black arrows). Note that in this case, the nonspecific staining is not also present in the PD-L1 slide (bottom) (20x magnification).

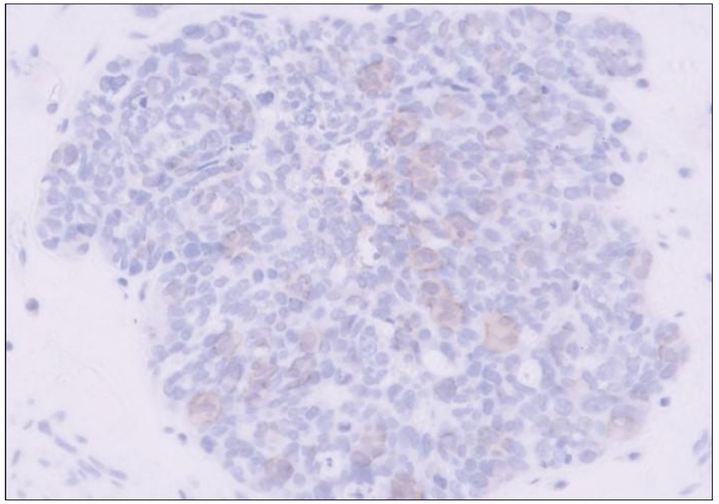


Figure 34b: EOC specimen stained with PD-L1 primary antibody exhibiting acceptable (≤ 1+ intensity) nonspecific DAB droplets staining (20× magnification).

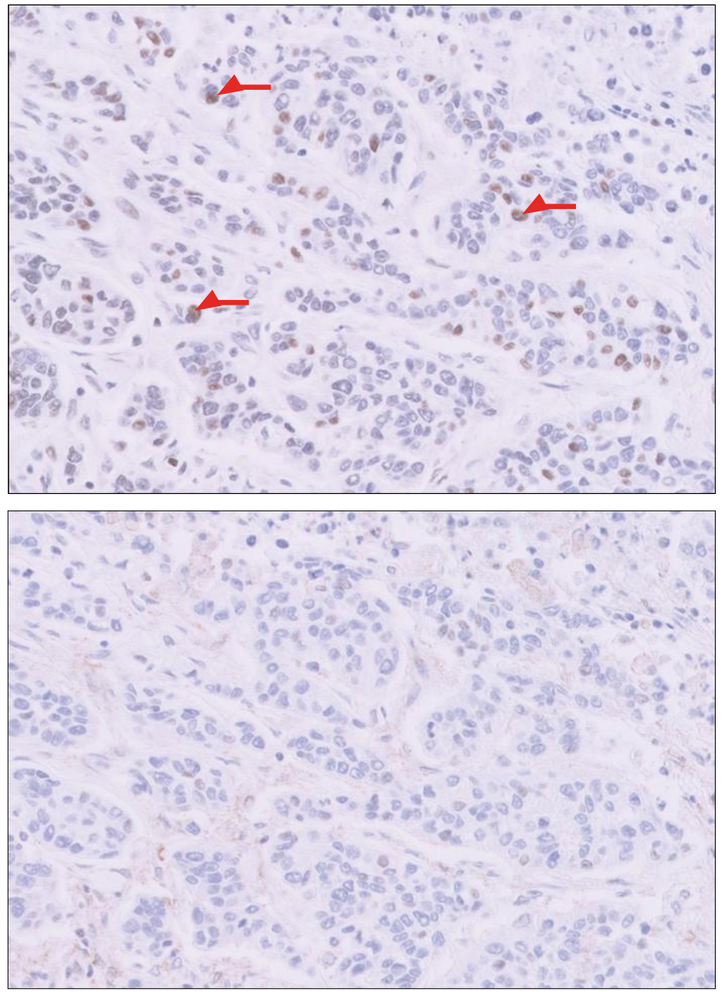


Figure 34c: Gastric or GEJ adenocarcinoma specimen exhibiting > 1+ nonspecific nuclear staining in the NCR-stained slide (top) (arrows) that is not also present in the PD-L1 stained slide (bottom). As the acceptance criteria for nonspecific staining, including nuclear staining, in the NCR and PD-L1 slides or NCR slide alone is ≤ 1+ intensity, the PD-L1 stained slide in this case should be considered non-evaluable (20× magnification).

Key point

All specimens must have ≤ 1+ nonspecific staining, including nuclear staining in scorable tumor regions

Folded Tissue

Areas of folded tissue can exhibit exaggerated staining and should be avoided while scoring (20× magnification).

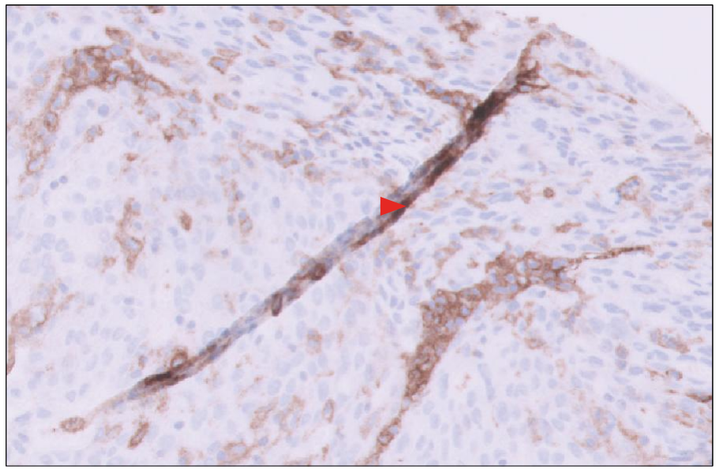


Figure 35: Folded tissue (arrow) may exhibit exaggerated PD-L1 staining; scoring of folded tissue areas should be avoided (20× magnification). Note: EOC specimen stained with PD-L1 primary antibody depicted.

Edge Artifact

Increased nonspecific staining that is observed around the periphery of the tissue specimen is known as the "edge artifact".

- Edge artifact is commonly due to drying of the tissue section after the 3-in-1 pretreatment procedure is performed (i.e., after loading on the Autostainer Link 48 and/or at any point during the staining procedure)
- If staining is only at the edge of the tissue section (i.e., a few cell layers of staining at the periphery and ending abruptly with penetration into the centrally located tumor), scoring at the edge of the tissue specimen should be avoided
- Inadequate processing of thick tissue samples may mimic edge artifact by rendering the central portion of the tissue suboptimally fixed relative to the peripheral areas. In these circumstances, the immunoreactivity based on the suboptimal central portion may be mistakenly interpreted as nonstaining because optimal fixation is only present at the periphery

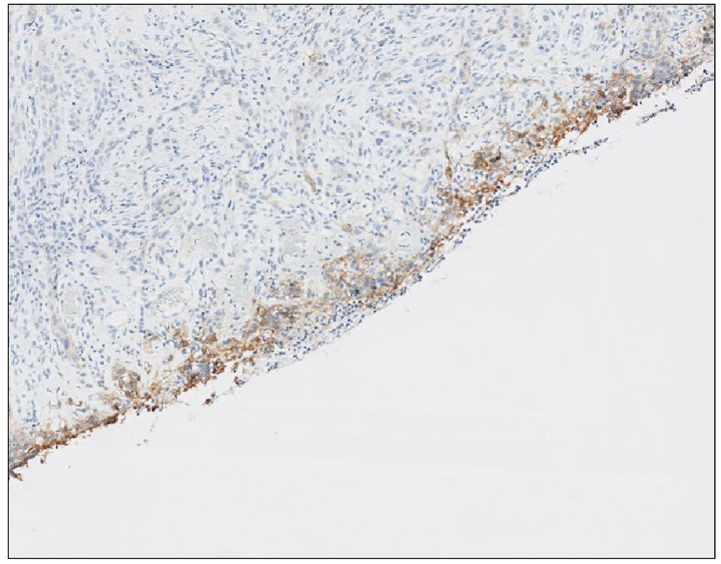


Figure 36: Gastric or GEJ adenocarcinoma specimen stained with PD-L1 primary antibody exhibiting edge artifact staining which should be excluded from scoring (5× magnification).

Key Point

Scoring of the edge of a specimen should be avoided if staining is inconsistent with the rest of the specimen and if it appears to be nonspecific in character

Ink Artifact

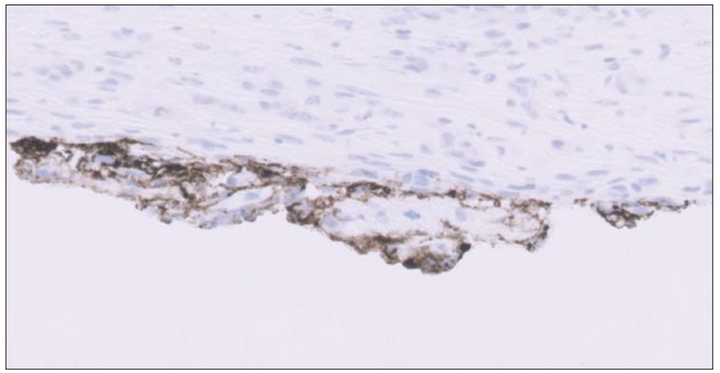


Figure 37: EOC specimen stained with PD-L1 primary antibody exhibiting ink artifact which should be ignored while scoring (20× magnification).

Key Point

Ink artifact should be ignored while scoring

Crush Artifact

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

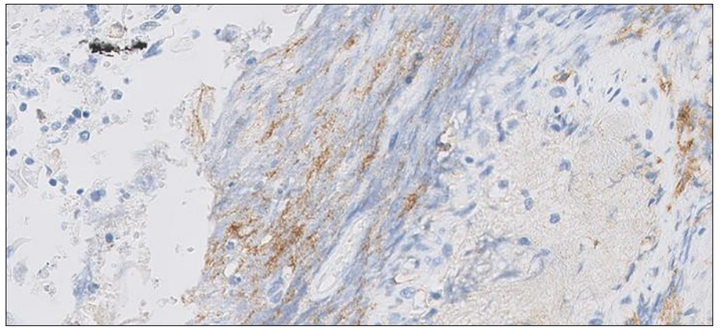


Figure 38: Esophageal carcinoma specimen stained with PD-L1 primary antibody exhibiting crush artifact; crush artifact should be excluded from scoring (20× magnification).

Key Point

Scoring of crush artifact should be avoided

Poor Fixation

Standardization of fixation is very important when using PD-L1 IHC 22C3 pharmDx, Code SK006. Suboptimal fixation of tissues may give erroneous results.

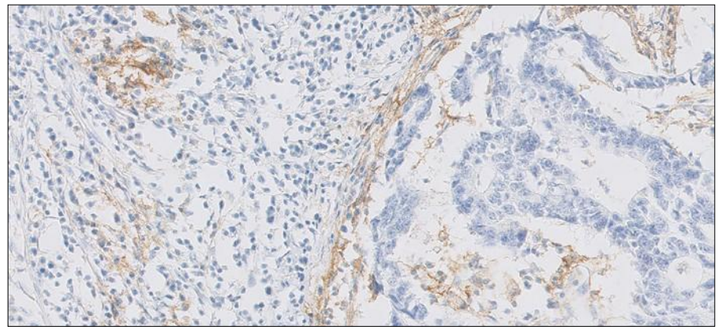


Figure 39: Esophageal carcinoma specimen stained with PD-L1 primary antibody exhibiting poor tissue fixation (20× magnification).

Key Point

Proper fixation is important for accurate PD-L1 assessment. Poorly fixed foci should be excluded from scoring

Hemosiderin

Hemosiderin pigment may be present in EOC tissue and will often exhibit a golden-brown color which may be confused with DAB staining. This pigment should be ignored and excluded from scoring. Utilization of the NCR slide can be useful for distinguishing hemosiderin pigment from DAB staining.

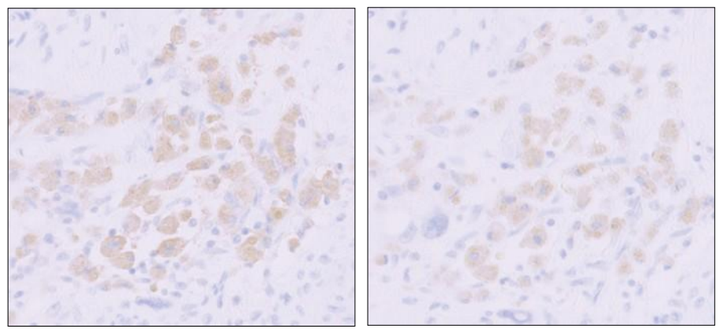


Figure 40: Macrophages containing hemosiderin pigment can occasionally appear to have DAB chromogen staining. The NCR can be helpful to ensure that these macrophages are excluded from the CPS numerator. Note: EOC specimen slide stained with PD-L1 primary antibody (left image) and NCR (right image) depicted (20× magnification).

Dystrophic Calcification

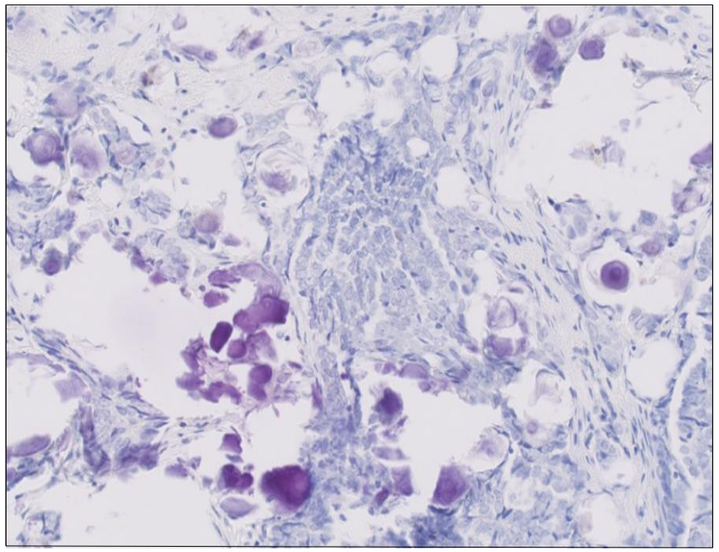


Figure 41: Dystrophic calcifications such as psammoma bodies are seen in EOC and should be excluded from scoring (20× magnification).

Necrotic cells and/or cellular debris and cystic fluid

Necrosis can be described as morphological changes indicative of cell death with undefined cellular detail. PD-L1 staining necrotic cells and/or cellular debris and cystic fluid may be present in EOC specimens and should be excluded from scoring.

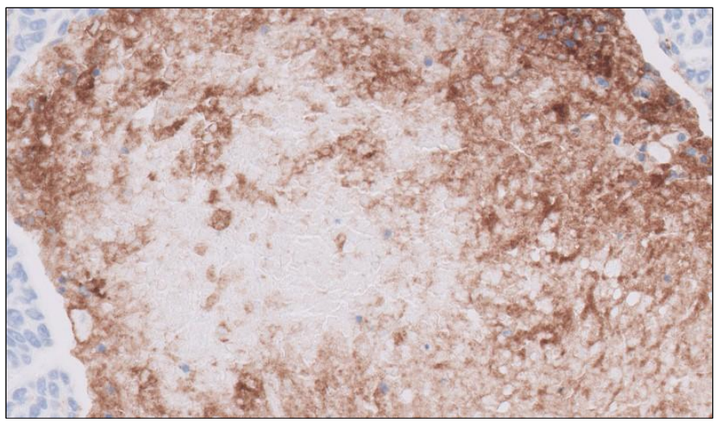


Figure 42: EOC specimen stained with PD-L1 primary antibody exhibiting staining of necrotic cells and cellular debris which should be excluded from scoring (20× magnification).

Key Point

PD-L1 staining necrotic cells and/or cellular debris and cystic fluid should be excluded from scoring

PD-L1 IHC 22C3 pharmDx, Code SK006 CPS 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.

CPS 0 Case Example

Case 1: CPS 0

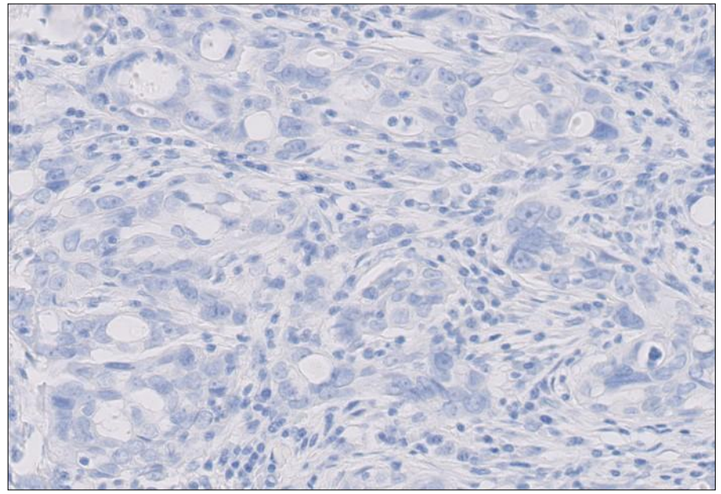


Figure 43: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 0 (20× magnification).

Near-Cutoff Negative Case Examples (CPS Range of Greater Than 0 but Less Than 1)

Case 2: Near-Cutoff Negative (CPS Range of Greater Than 0 but Less Than 1)

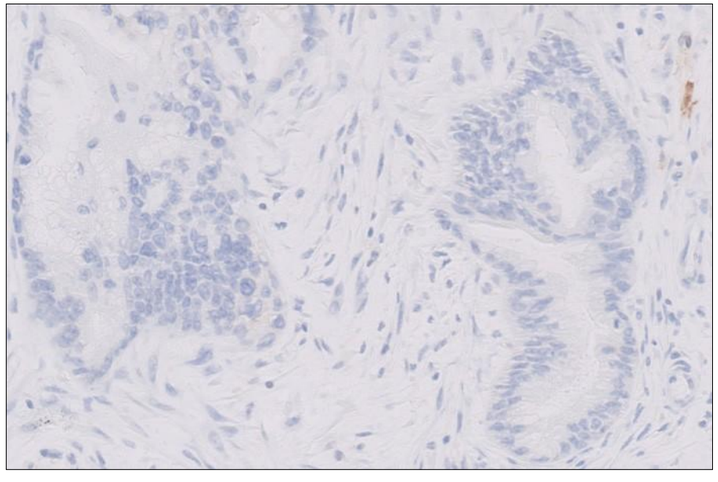


Figure 44: EOC specimen stained with PD-L1 primary antibody exhibiting a PD-L1 expression level of CPS < 1 (20× magnification). Note: Although this field contains PD-L1 staining cells, their numbers are not high enough to bring the score to CPS 1. The CPS should always be reported as a whole number (no fractions). Therefore, the score for this case should be reported as CPS 0.

Case 3: Near-Cutoff Negative (CPS Range of Greater Than 0 but Less Than 1)

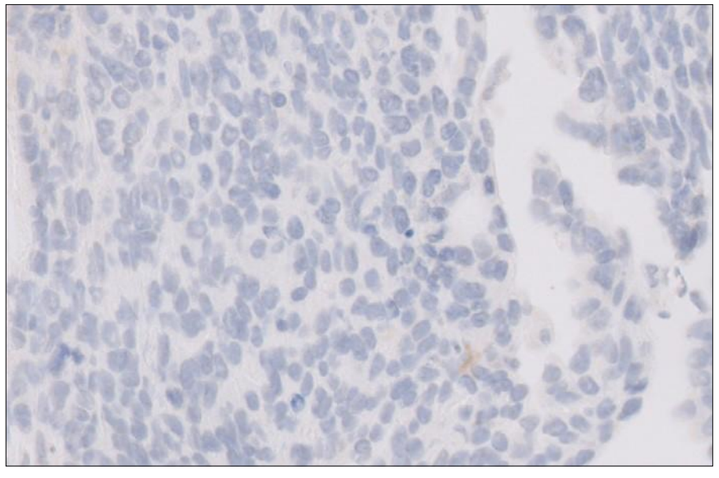


Figure 45: EOC specimen stained with PD-L1 primary antibody exhibiting a PD-L1 expression level of CPS < 1 (20× magnification). Note: Although this field contains PD-L1 staining cells, their numbers are not high enough to bring the score to CPS 1. The CPS should always be reported as a whole number (no fractions). Therefore, the score for this case should be reported as CPS 0.

Case 4: Near-Cutoff Negative (CPS Range of Greater Than 0 but Less Than 1)

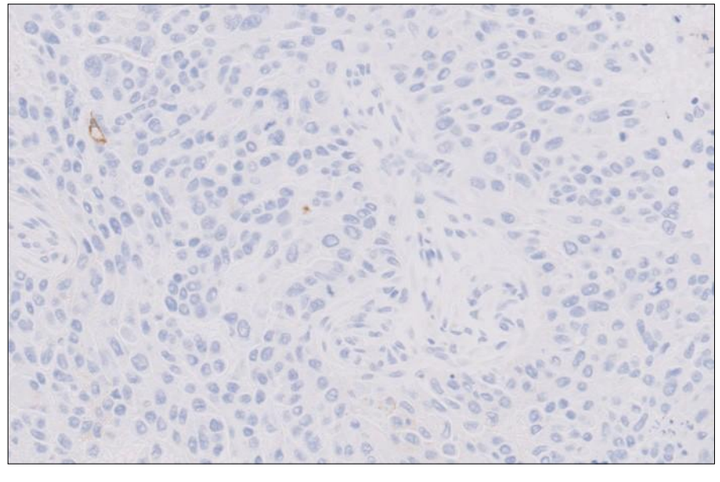


Figure 46: EOC specimen stained with PD-L1 primary antibody exhibiting a PD-L1 expression level of CPS < 1 (20× magnification). Note: Although this field contains PD-L1 staining cells, their numbers are not high enough to bring the score to CPS 1. The CPS should always be reported as a whole number (no fractions). Therefore, the score for this case should be reported as CPS 0.

Case 5: Near-Cutoff Negative (CPS Range of Greater Than 0 but Less Than 1)

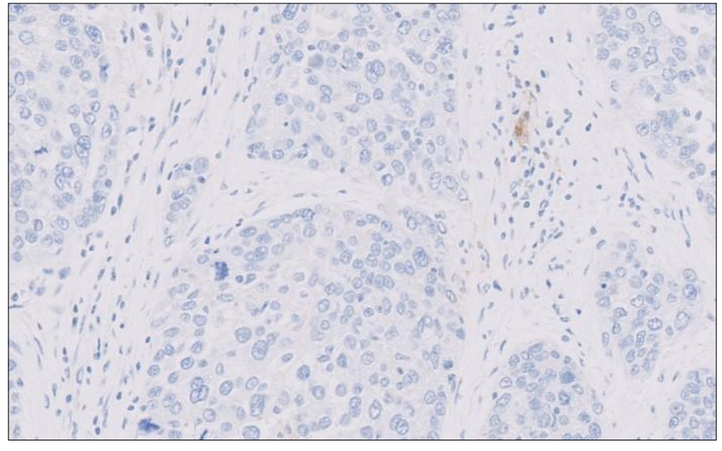


Figure 47: EOC specimen stained with PD-L1 primary antibody exhibiting a PD-L1 expression level of CPS < 1 (20× magnification). Note: Although this field contains PD-L1 staining cells, their numbers are not high enough to bring the score to CPS 1. The CPS should always be reported as a whole number (no fractions). Therefore, the score for this case should be reported as CPS 0.

Case 6: Near-Cutoff Negative (CPS Range of Greater Than 0 but Less Than 1)

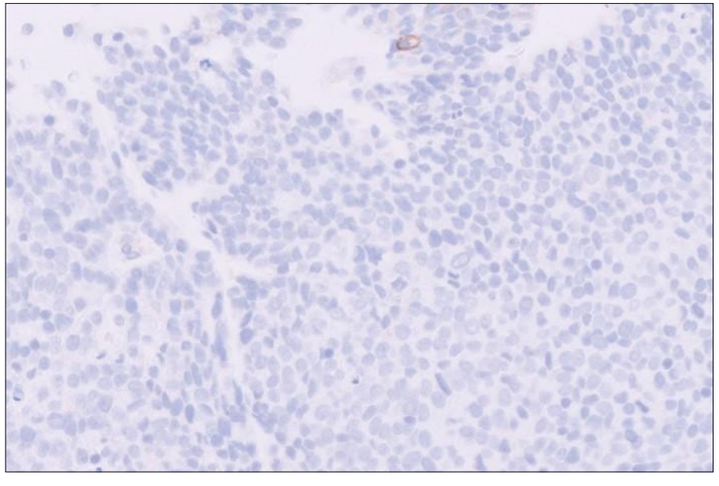


Figure 48: EOC specimen stained with PD-L1 primary antibody exhibiting a PD-L1 expression level of CPS < 1 (20× magnification). Note: Although this field contains PD-L1 staining cells, their numbers are not high enough to bring the score to CPS 1. The CPS should always be reported as a whole number (no fractions). Therefore, the score for this case should be reported as CPS 0.

Case 7: Near-Cutoff Negative (CPS Range of Greater Than 0 but Less Than 1

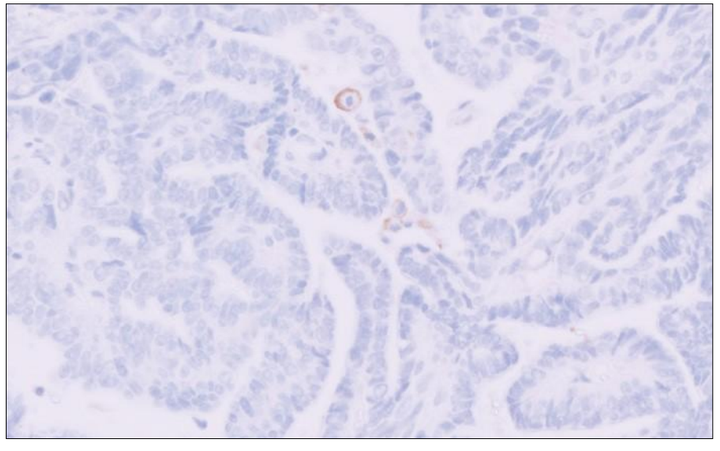


Figure 49: EOC specimen stained with PD-L1 primary antibody exhibiting a PD-L1 expression level of CPS < 1 (20× magnification). Note: Although this field contains PD-L1 staining cells, their numbers are not high enough to bring the score to CPS 1. The CPS should always be reported as a whole number (no fractions). Therefore, the score for this case should be reported as CPS 0.

Near-Cutoff Positive Case Examples (CPS Range 1–10)

Case 8: Near-Cutoff Negative (CPS Range of Greater Than 0 but Less Than 1)

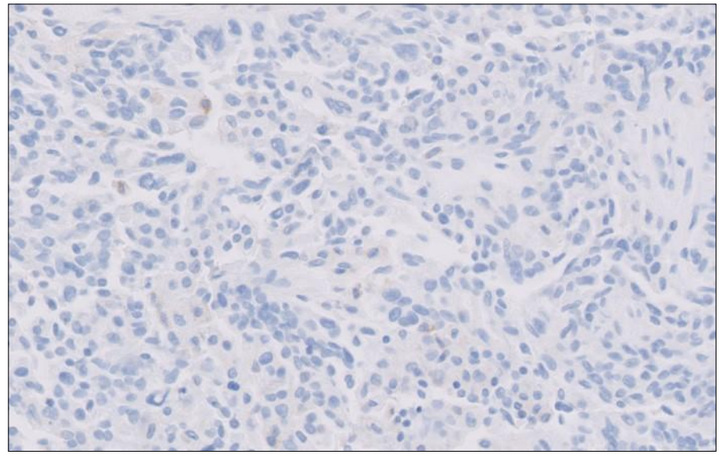


Figure 50: EOC specimen stained with PD-L1 primary antibody exhibiting a PD-L1 expression level of CPS < 1 (20× magnification). Note: Although this field contains PD-L1 staining cells, their numbers are not high enough to bring the score to CPS 1. The CPS should always be reported as a whole number (no fractions). Therefore, the score for this case should be reported as CPS 0.

Case 9: Near-Cutoff Positive (CPS Range 1–10)

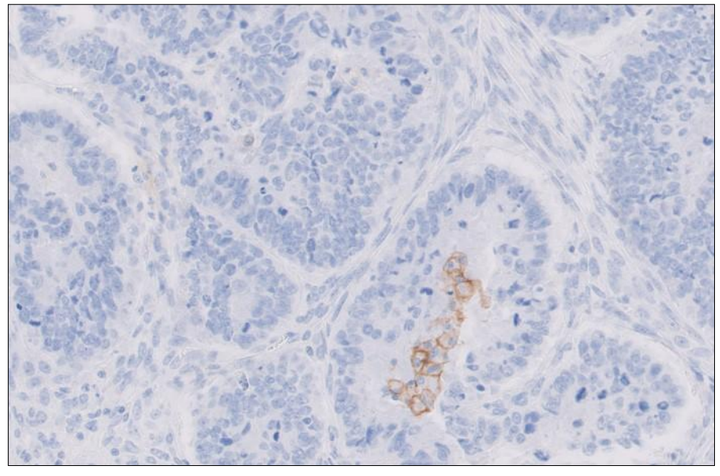


Figure 51: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 2, however any numerical CPS between 1–3 could be assigned to this image (20× magnification).

Case 10: Near-Cutoff Positive (CPS Range 1–10)

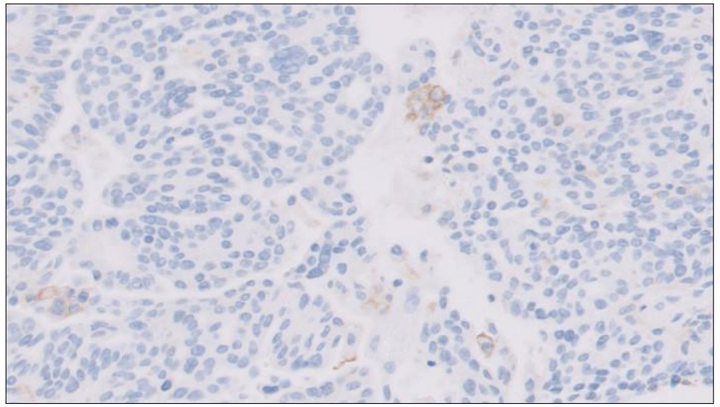


Figure 52: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 2, however any numerical CPS between 1–4 could be assigned to this image (20× magnification).

Case 11: Near-Cutoff Positive (CPS Range 1–10)

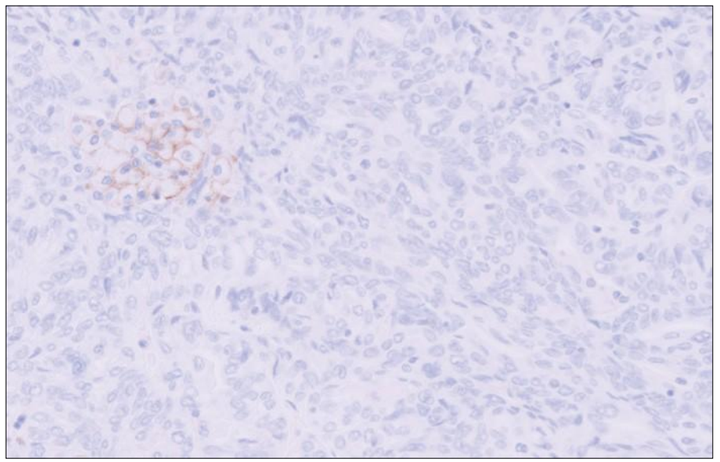


Figure 53: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 4, however any numerical CPS between 3–5 could be assigned to this image (20× magnification).

Case 12: Near-Cutoff Positive (CPS Range 1–10)

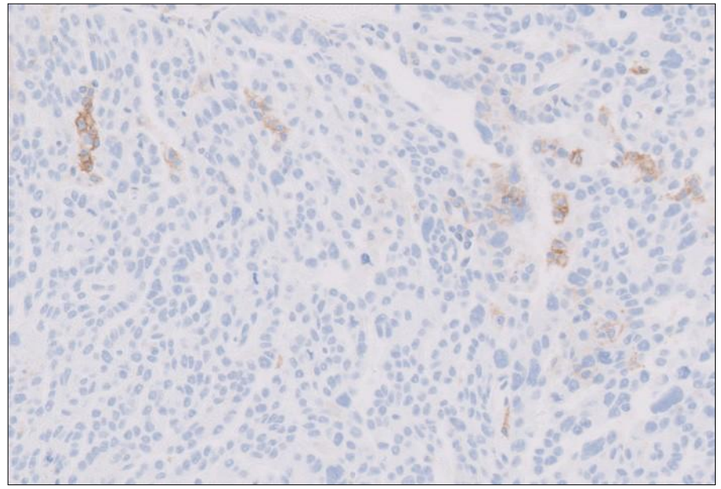


Figure 54: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 4, however any numerical CPS between 3–5 could be assigned to this image (20× magnification).

Case 13: Near-Cutoff Positive (CPS Range 1–10)

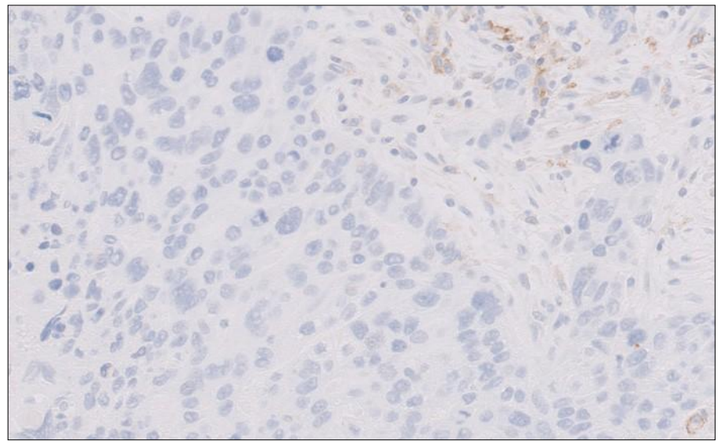


Figure 55: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 4, however any numerical CPS between 3–5 could be assigned to this image (20× magnification).

Case 14: Near-Cutoff Positive (CPS Range 1–10)

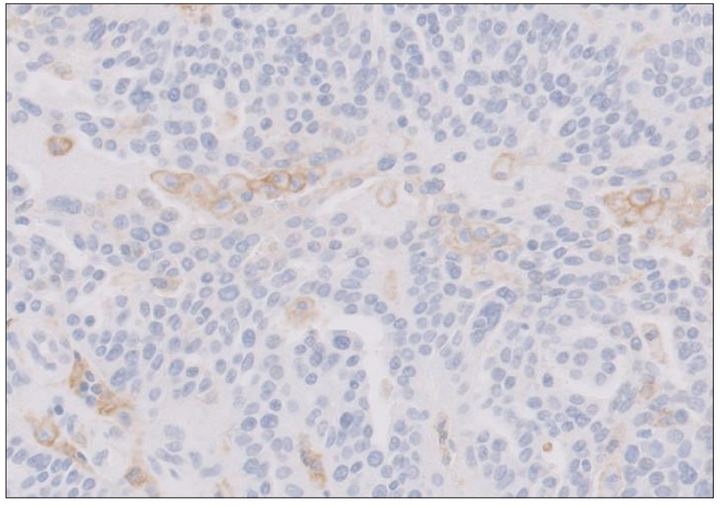


Figure 56: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 5, however any numerical CPS between 4–6 could be assigned to this image (20× magnification).

Case 15: Near-Cutoff Positive (CPS Range 1–10)

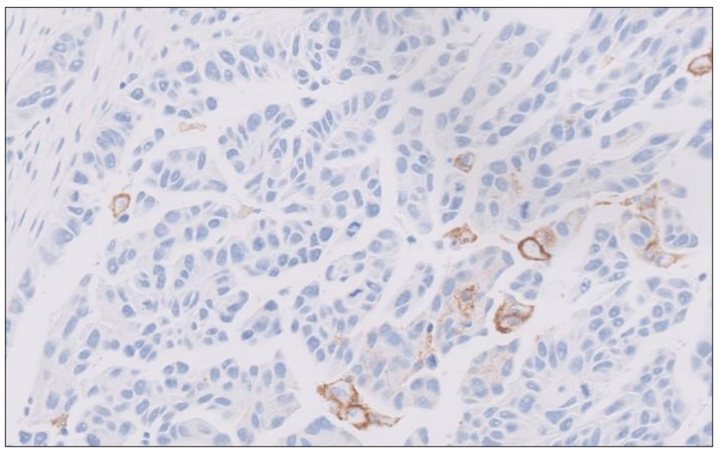


Figure 57: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 5, however any numerical CPS between 4–6 could be assigned to this image (20× magnification).

CPS ≥ 1 Case Examples

Case 16: Near-Cutoff Positive (CPS Range 1–10)

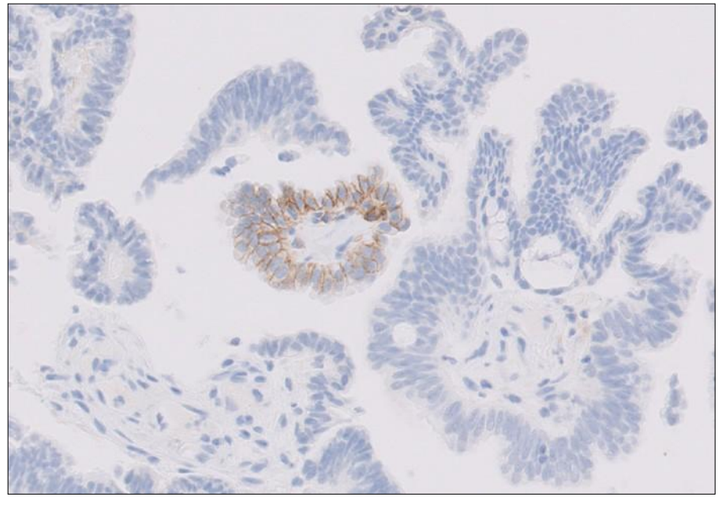


Figure 58: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 7, however any numerical CPS between 6–8 could be assigned to this image (20× magnification).

Case 17: **CPS ≥1**

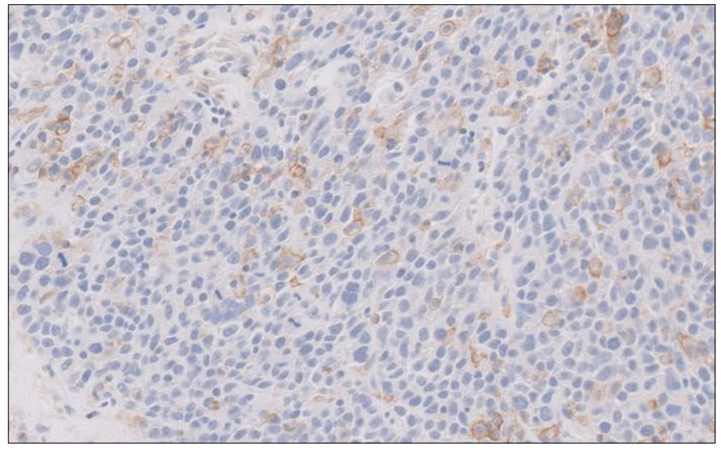


Figure 59: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 14, however any numerical CPS between 11–16 could be assigned to this image (20× magnification).

Case 18: CPS ≥ 1

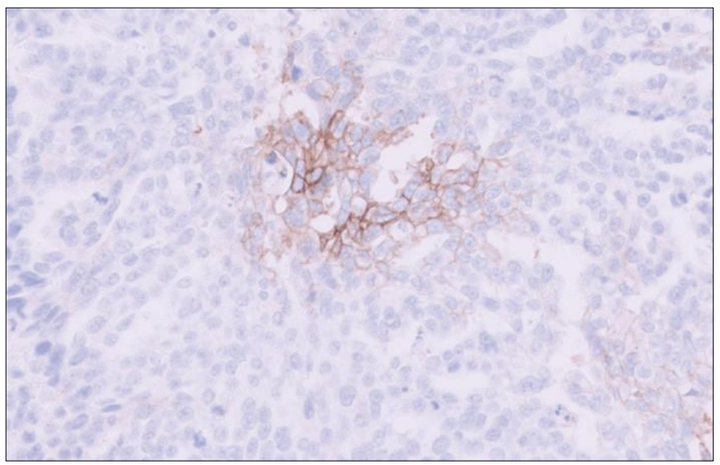


Figure 60: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 16, however any numerical CPS between 14–18 could be assigned to this image (20× magnification).

Case 19: CPS ≥ 1

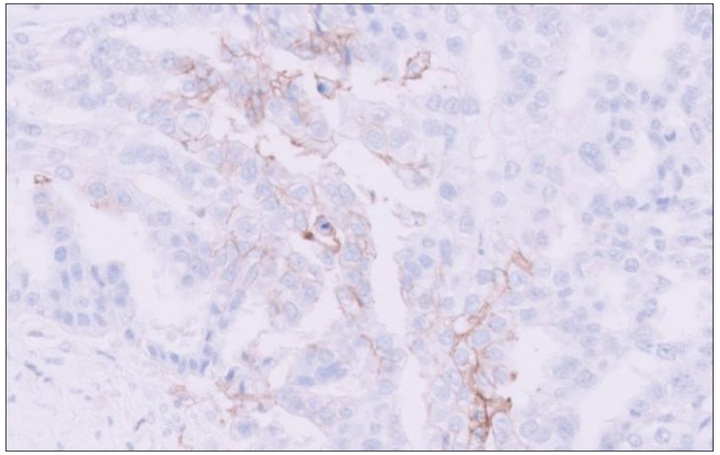


Figure 61: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 27, however any numerical CPS between 24–30 could be assigned to this image (20× magnification).

Case 20: CPS ≥ 1

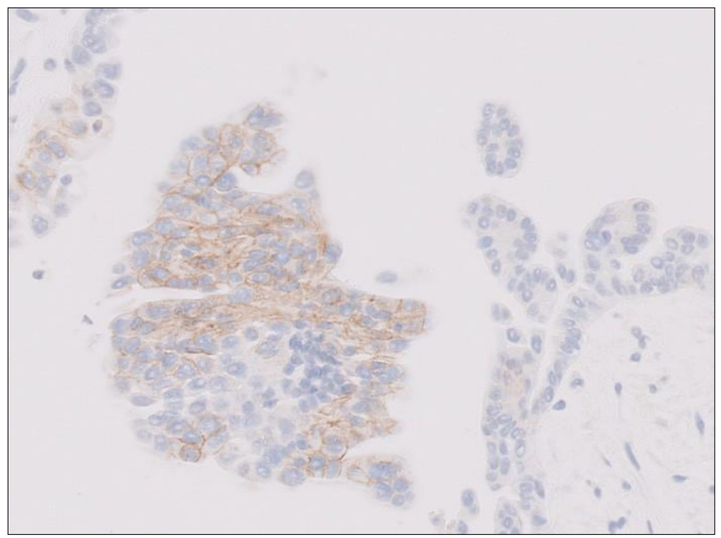


Figure 62: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 40, however any numerical CPS between 36–44 could be assigned to this image (20× magnification).

Case 21: CPS ≥ 1

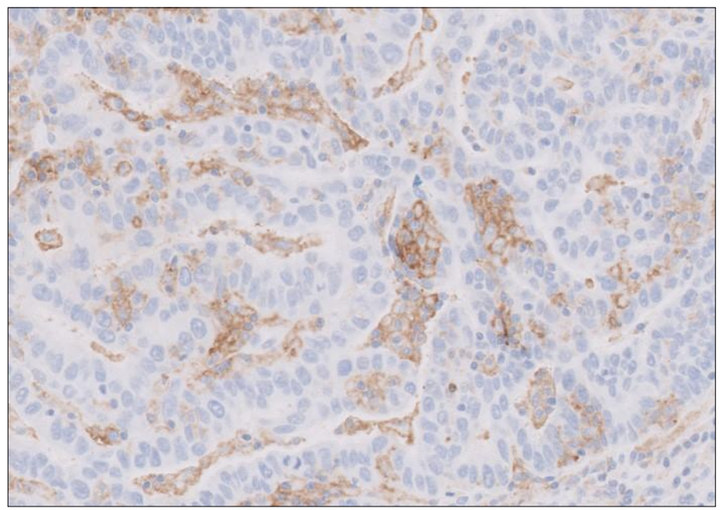


Figure 63: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 40, however any numerical CPS between 30–45 could be assigned to this image (20× magnification).

Case 22: CPS ≥ 1

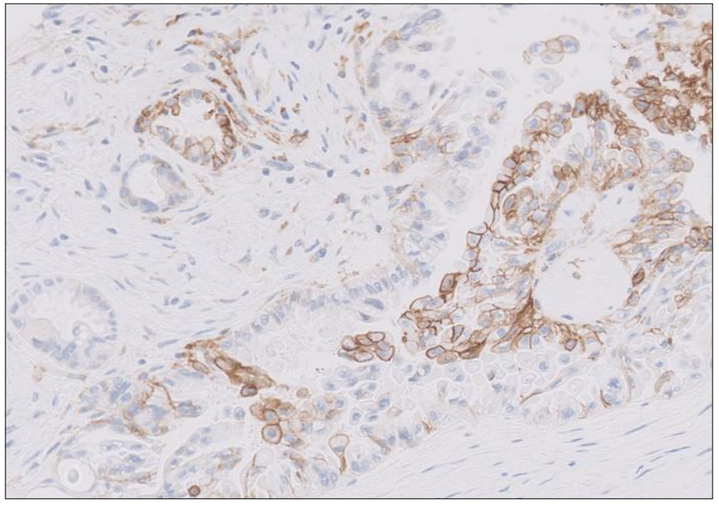


Figure 64: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 51, however any numerical CPS between 45–55 could be assigned to this image (20× magnification).

Case 23: CPS ≥ 1

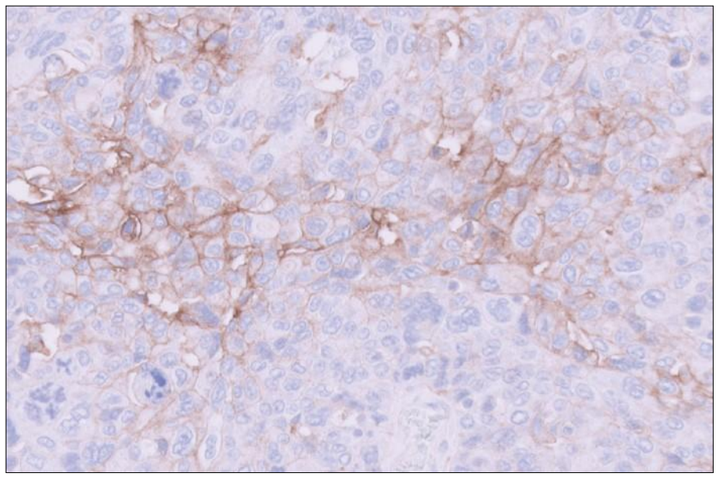


Figure 65: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 60, however any numerical CPS between 55–70 could be assigned to this image (20× magnification).

Case 24: CPS ≥ 1

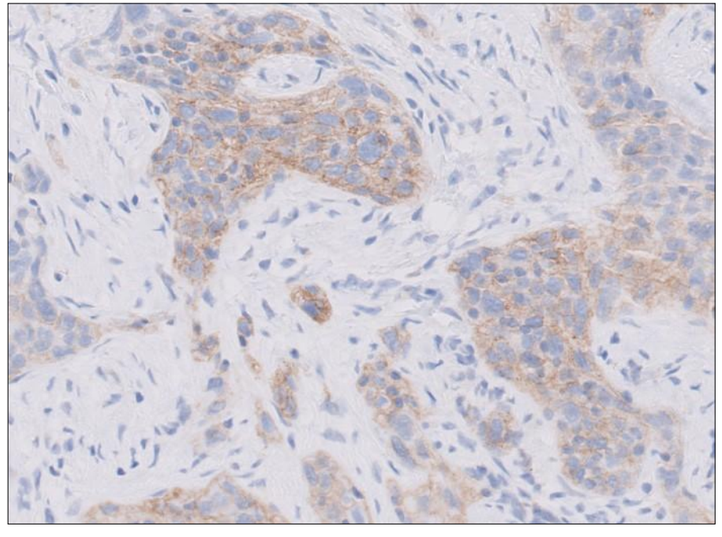


Figure 66: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 79, however any numerical CPS between 75–85 could be assigned to this image (20× magnification).

Case 25: CPS ≥ 1

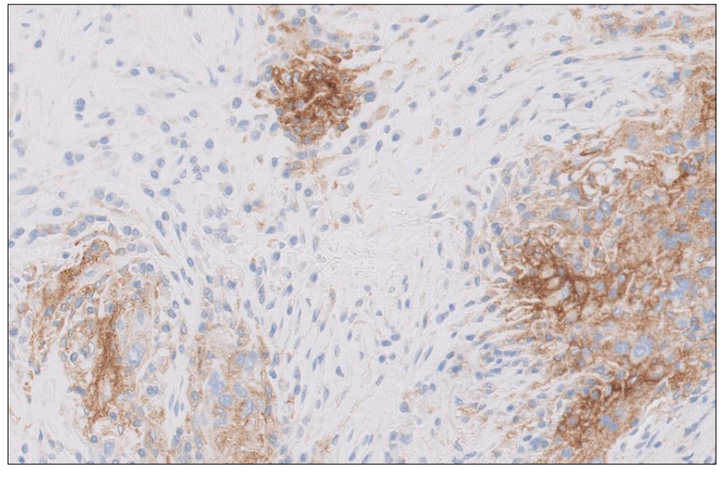


Figure 67: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 100 (20× magnification).

Case 26: CPS ≥ 1

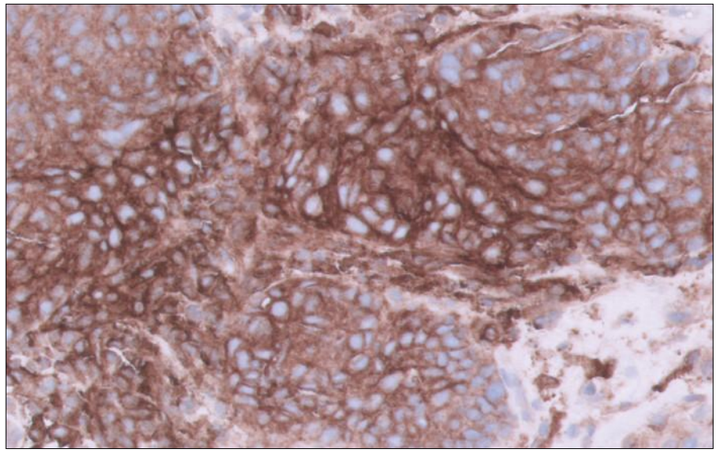


Figure 68: EOC specimen stained with PD-L1 primary antibody exhibiting a CPS of 100 (20× magnification).

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 ≥ 1+ intensity within the boundaries of the MCF-7 cell pellet are acceptable

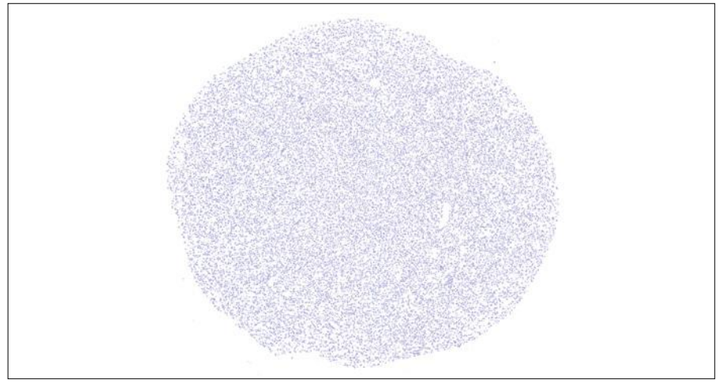


Figure 69: Ideal MCF-7 cell pellet (2× magnification).

Passing PD-L1 Positive CCL

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

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

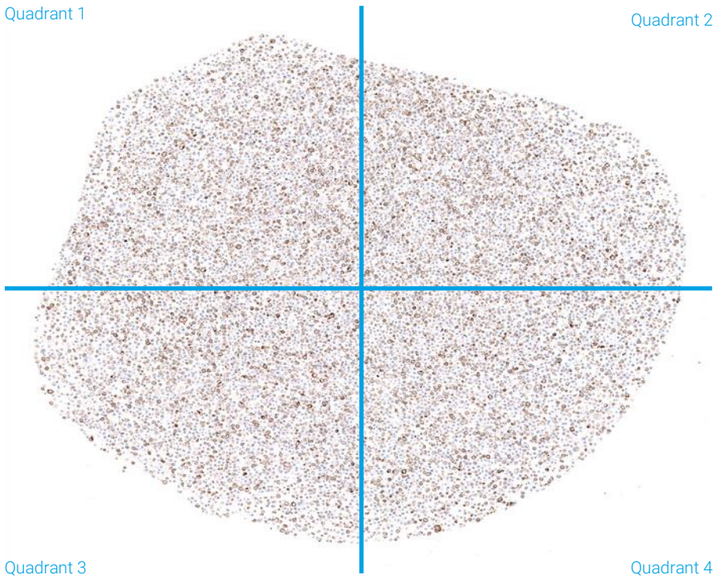
Borderline Passing vs. Passing PD-L1 Positive CCL

Borderline Passing PD-L1 positive CCL

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

Evaluation Strategy for Borderline Passing PD-L1 Positive CCL

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



Quadrant 1

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

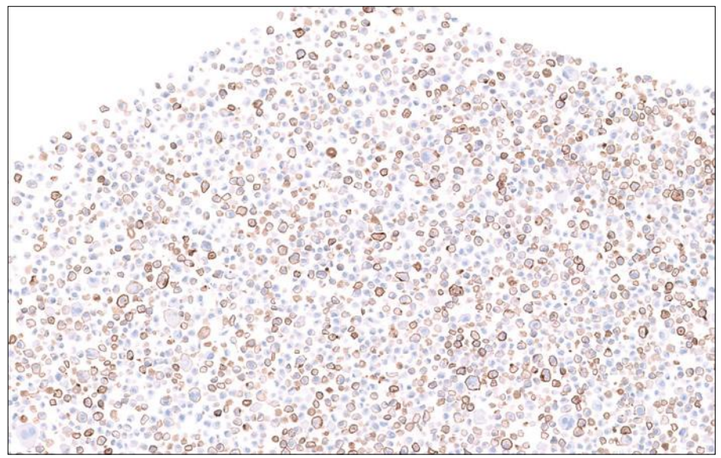


Figure 72: NCI-H226 cell pellet (5× magnification).

Quadrant 2

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

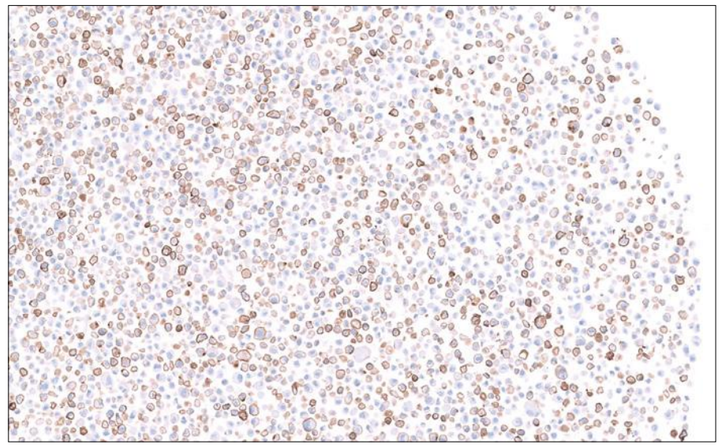


Figure 73: NCI-H226 cell pellet (5× magnification).

Quadrant 3

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

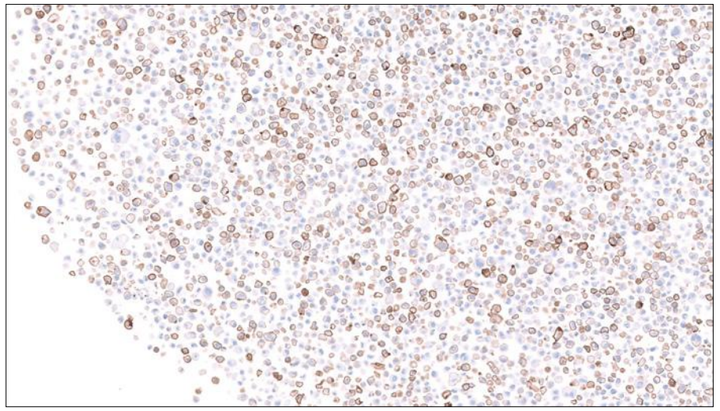


Figure 74: NCI-H226 cell pellet (5× magnification).

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 ≥ 2+.

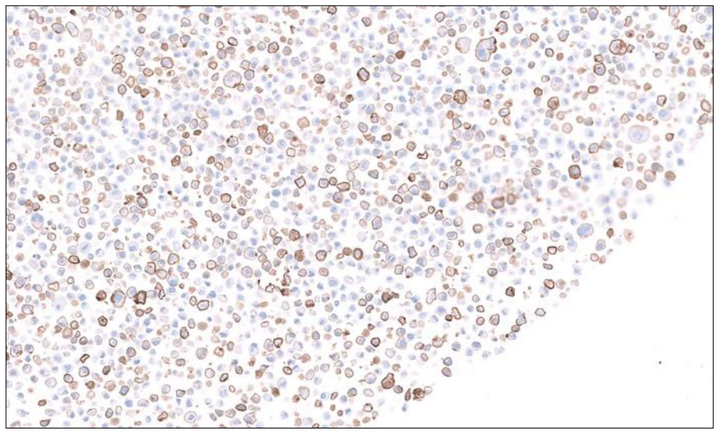
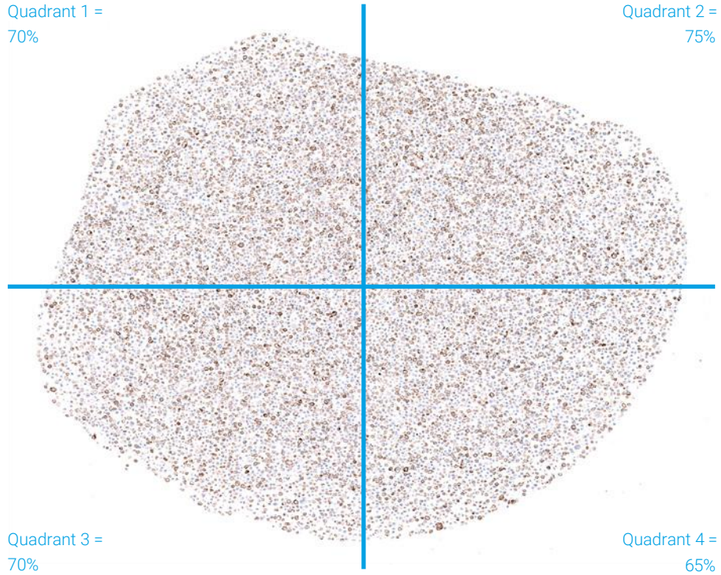


Figure 75: NCI-H226 cell pellet (5× magnification).

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 ≥ 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 ≥ 2+

NCI-H226 positive control cell pellet meets acceptance criteria.

Failed CCL

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

Passing PD-L1 negative CCL

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

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

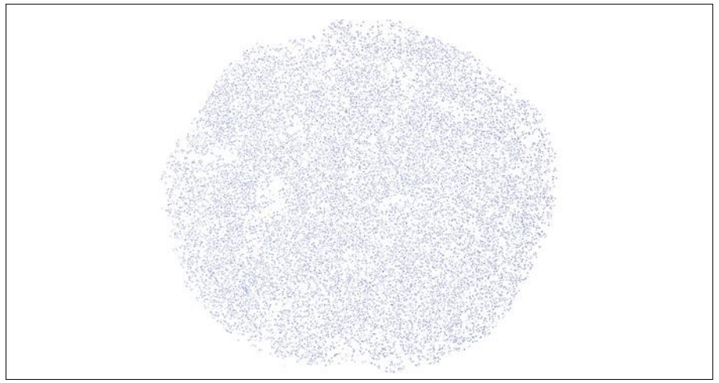


Figure 76: MCF-7 cell pellet (2× magnification).

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

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

Failed PD-L1 positive CCL (10×)

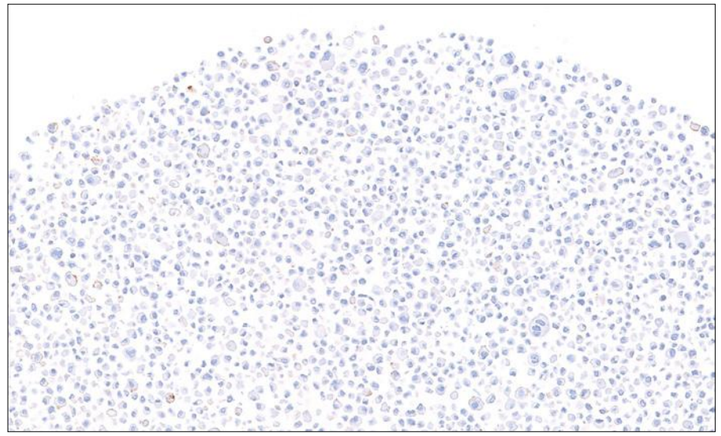


Figure 78: NCI-H226 cell pellet (10× magnification).

Failed PD-L1 positive CCL (20×)

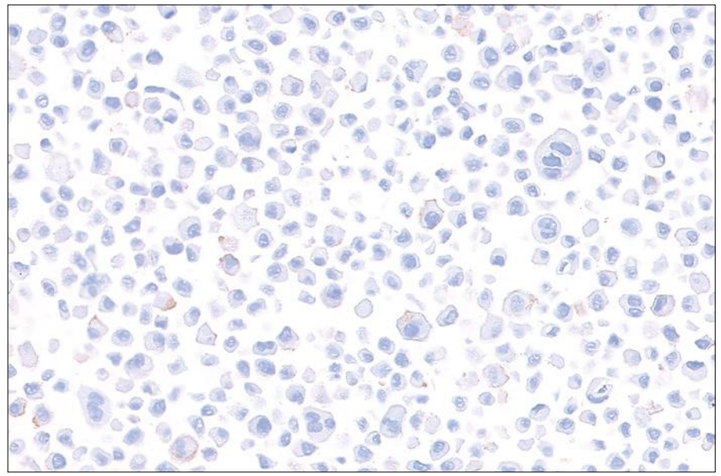


Figure 79: NCI-H226 cell pellet (20× magnification).

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

Passing PD-L1 negative CCL

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

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

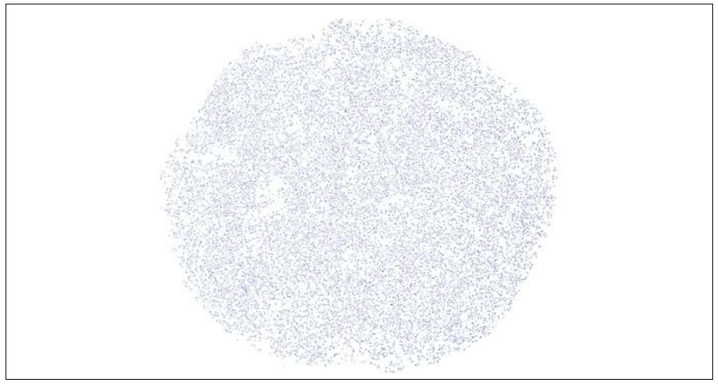


Figure 80: MCF-7 cell pellet (2× magnification).

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

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

Failed PD-L1 positive CCL (10×)

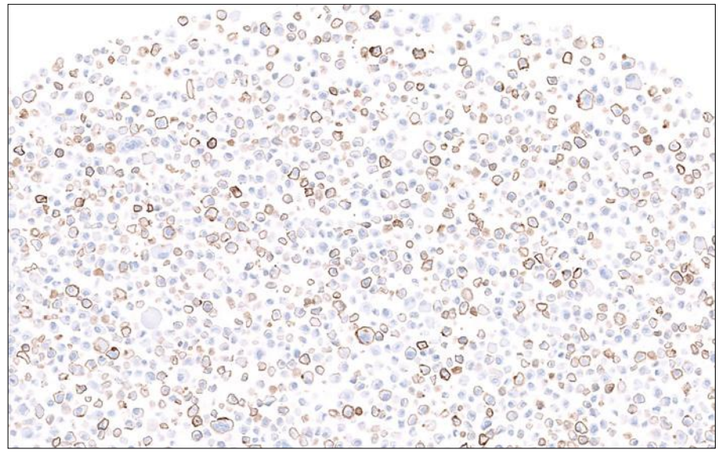


Figure 82: NCI-H226 cell pellet (10× magnification).

Failed PD-L1 positive CCL (20×)

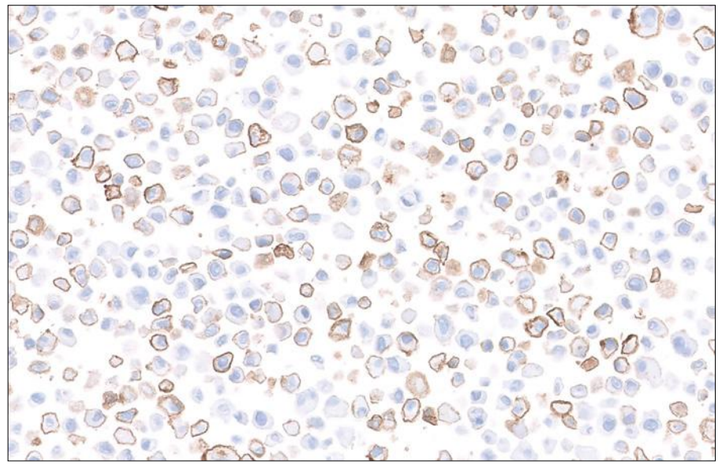


Figure 83: NCI-H226 cell pellet (20× magnification).

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 of cells with membrane staining
- Nonspecific staining < 1+

Figure 84: NCI-H226 cell pellet (2× magnification).

Failed PD-L1 negative CCL

- Nonspecific (nuclear) staining is ≥ 1+ staining intensity
- There are > 10 total cells with distinct cell membrane and/or nonspecific staining that is ≥ 1+ intensity

Figure 85: MCF-7 cell pellet (2× magnification).

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

Failed PD-L1 negative CCL (10×)

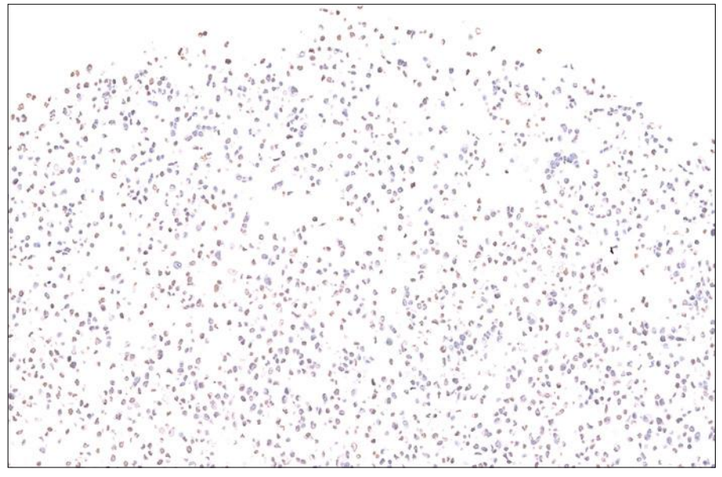


Figure 86: MCF-7 cell pellet (10× magnification).

Failed PD-L1 negative CCL (20×)

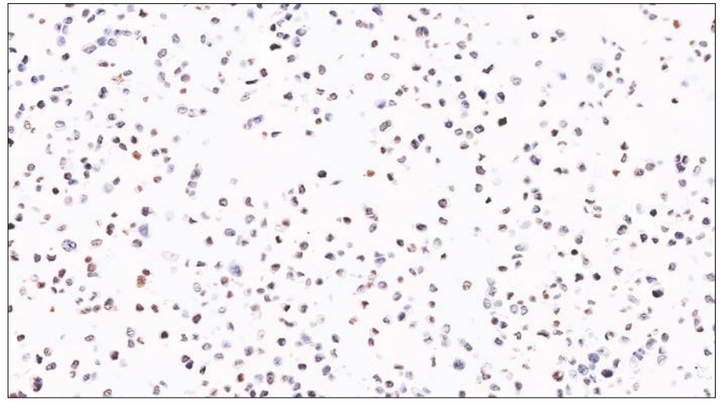


Figure 87: MCF-7 cell pellet (20× magnification).

Troubleshooting Guide

Troubleshooting Guidelines for PD-L1 IHC 22C3 pharmDx, Code SK006

Refer to the current IFU for PD-L1 IHC 22C3 pharmDx, Code SK006 troubleshooting guidelines.

References

1. PD-L1 IHC 22C3 pharmDx, Code SK006 [Instructions for Use]. Santa Clara, CA: Agilent Technologies, Inc.; 2026.
2. KEYTRUDA® (pembrolizumab) [package insert]. Merck Sharp & Dohme LLC, Rahway, NJ, USA; 2026.

Notes

For PD-L1 testing,

Choose PD-L1 IHC 22C3 pharmDx, Code SK006– the ONE Leading Assay with KEYTRUDA® (pembrolizumab)

KEYNOTE

The **ONE** PD-L1 assay used in KEYTRUDA® (pembrolizumab) clinical trials[a,b]

The **ONE** PD-L1 assay first FDA-approved with KEYTRUDA® (pembrolizumab) in every indication that requires PD-L1 testing[a,b]

The **ONE** PD-L1 assay trusted worldwide to test hundreds of thousands of patients for KEYTRUDA® (pembrolizumab)[a]

a. KEYTRUDA® (pembrolizumab) [package insert]. Merck Sharp & Dohme LLC, Rahway, NJ, USA; 2026. **b.** PD-L1 IHC 22C3 pharmDx, Code SK006 [Instructions for Use]. Santa Clara, CA: Agilent Technologies, Inc.; 2026.

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For countries outside of the United States, see the local KEYTRUDA® (pembrolizumab) product label for approved indications and PD-L1 expression cutoff values to guide therapy.

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