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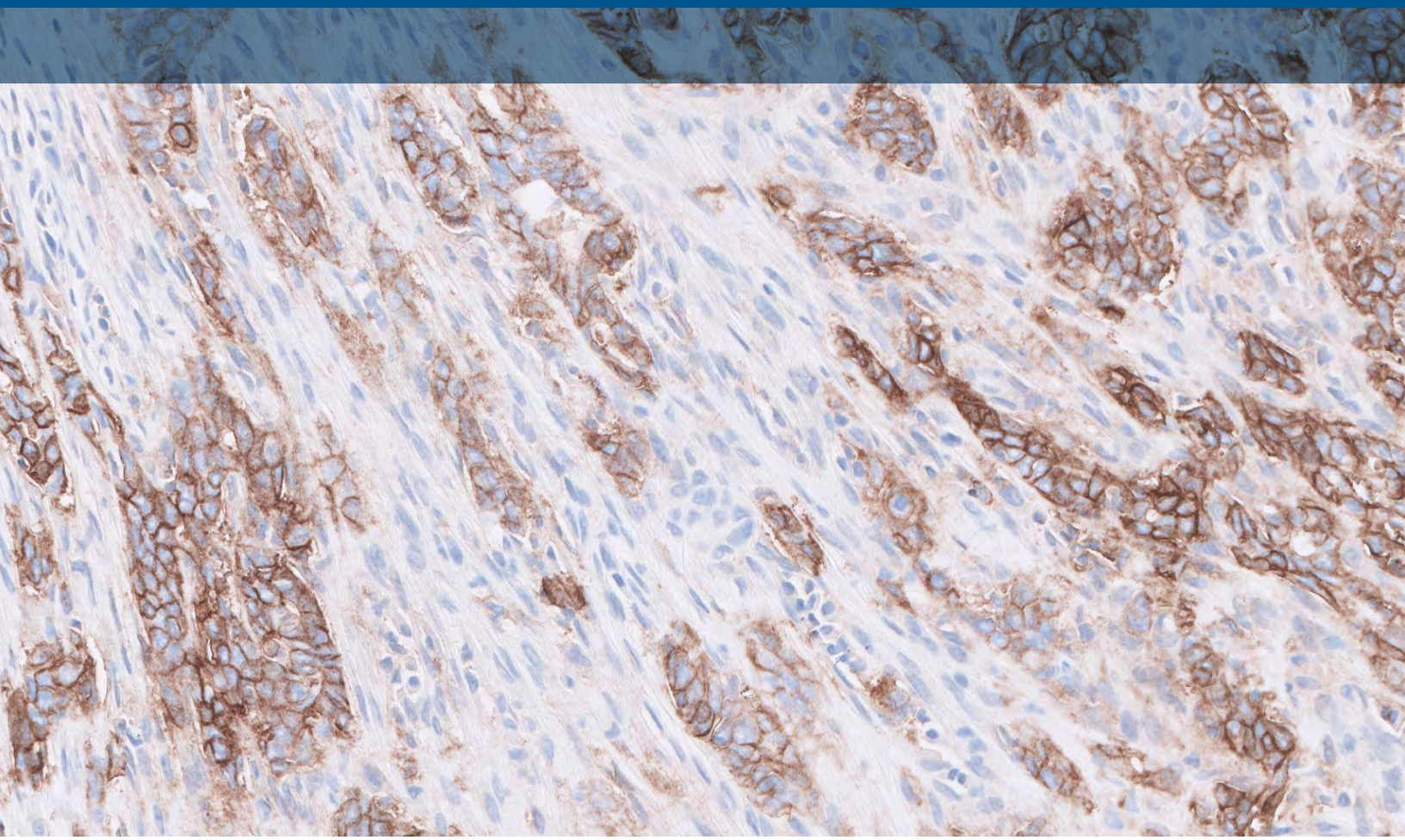
PD-L1 IHC 28-8 pharmDx Interpretation Manual—Gastric Adenocarcinoma, Gastroesophageal Junction (GEJ) Adenocarcinoma, and Esophageal Adenocarcinoma

PD-L1 IHC 28-8 pharmDx is CE-IVD marked for in vitro diagnostic use



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Check the local OPDIVO® product label for approved indications and expression cut-off values to guide therapy.

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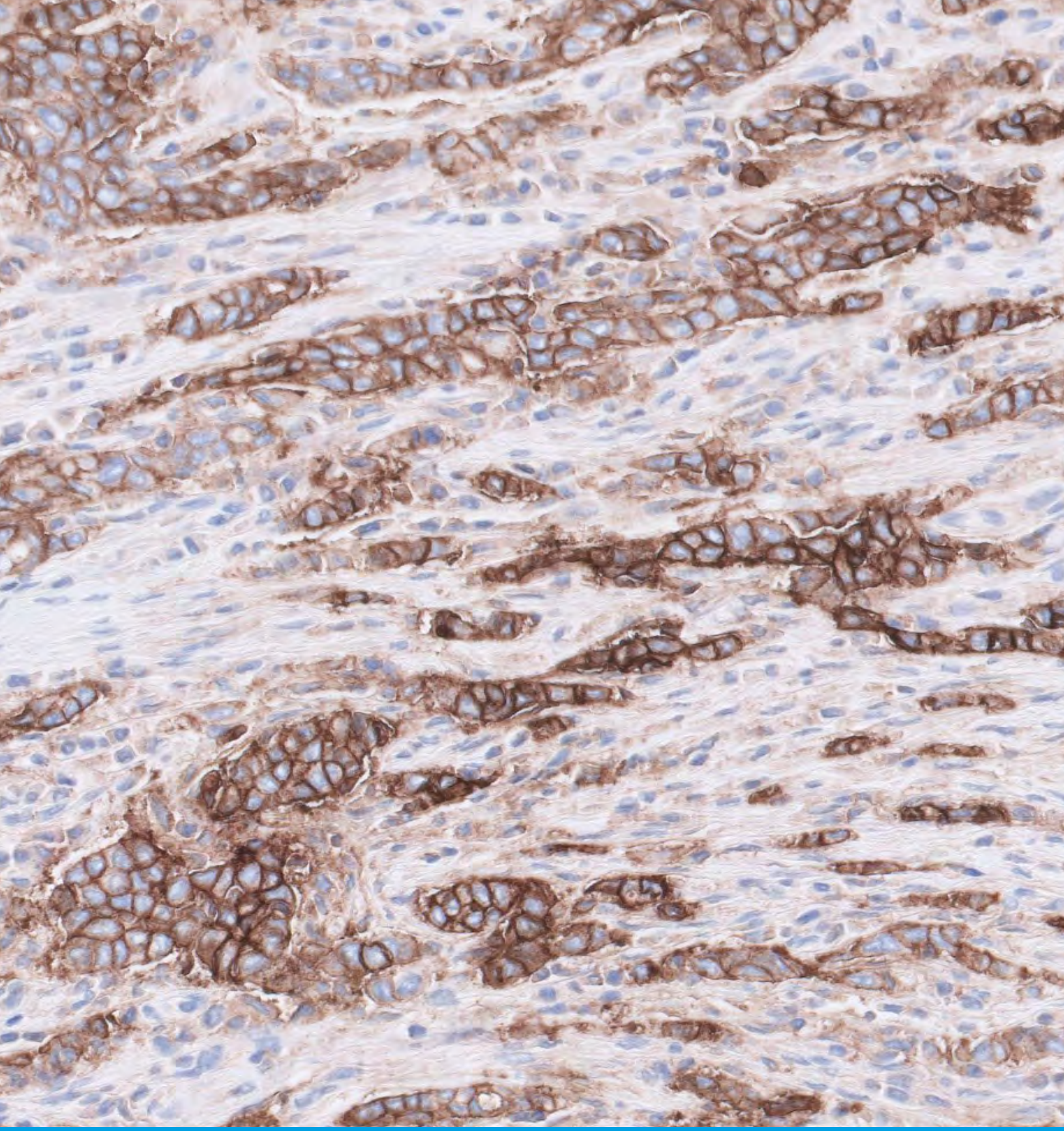


PD-L1 IHC 28-8 pharmDx
Interpretation Manual - Gastric, GEJ, and Esophageal Adenocarcinoma

Introduction

Intended Use

For In Vitro Diagnostic Use

PD-L1 IHC 28-8 pharmDx is a qualitative immunohistochemical assay using Monoclonal Rabbit Anti-PD-L1, Clone 28-8 intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) non-squamous non-small cell lung cancer (nsNSCLC), squamous cell carcinoma of the head and neck (SCCHN), urothelial carcinoma (UC), melanoma, gastric adenocarcinoma, gastroesophageal junction (GEJ) adenocarcinoma, and esophageal adenocarcinoma tissues using EnVision FLEX visualization system on Autostainer Link 48.

PD-L1 protein expression in nsNSCLC, SCCHN, UC, and melanoma is determined by using % tumor cell expression, which is the percentage of evaluable tumor cells exhibiting partial or complete membrane staining at any intensity.

PD-L1 protein expression in gastric adenocarcinoma, GEJ adenocarcinoma, and esophageal adenocarcinoma 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.

Companion Diagnostic Indications

Tumor Indication	PD-L1 Expression Clinical Cut-off	Intended Use
Gastric, GEJ, or Esophageal Adenocarcinoma	CPS ≥ 5	PD-L1 IHC 28-8 pharmDx is indicated as an aid in identifying gastric, gastroesophageal junction, or esophageal adenocarcinoma patients for treatment with OPDIVO® (nivolumab) in combination with fluoropyrimidine and platinum-based chemotherapy.

PD-L1 expression (≥ 1% or ≥ 5% or ≥ 10% tumor cell expression) as detected by PD-L1 IHC 28-8 pharmDx in non-squamous NSCLC (nsNSCLC) may be associated with enhanced survival from OPDIVO® (nivolumab).

PD-L1 expression (≥ 1% tumor cell expression) as detected by PD-L1 IHC 28-8 pharmDx in SCCHN may be associated with enhanced survival from OPDIVO® (nivolumab).

PD-L1 expression (≥ 1% tumor cell expression) as detected by PD-L1 IHC 28-8 pharmDx in urothelial carcinoma may be associated with enhanced response rate from OPDIVO®.

PD-L1 expression (≥ 1% or ≥ 5% tumor cell expression) as detected by PD-L1 IHC 28-8 pharmDx in melanoma may be used as an aid in the assessment of patients for whom OPDIVO® (nivolumab) and YERVOY® (ipilimumab) combination treatment is being considered.

See the OPDIVO® and YERVOY® product labels for specific clinical circumstances guiding PD-L1 testing.

CheckMate-649 results highlight overall survival (OS) benefit from OPDIVO® (nivolumab) in combination with fluoropyrimidine and platinum-based chemotherapy for gastric, GEJ, and esophageal adenocarcinoma patients whose tumors express PD-L1 with a Combined Positive Score (CPS) ≥ 5.

PD-L1 IHC 28-8 pharmDx Interpretation Manual - Overview

This gastric, GEJ, and esophageal adenocarcinoma PD-L1 IHC 28-8 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, gastric, GEJ, and esophageal adenocarcinoma. PD-L1 expression evaluation may be used to identify patients for anti-PD-1 immunotherapy.

This manual provides detailed scoring guidelines and technical information from the PD-L1 IHC 28-8 pharmDx Instructions for Use (IFU) to ensure high-quality staining and diagnostic assessment. To help familiarize you with the requirements for scoring gastric, GEJ, and esophageal adenocarcinoma specimens stained with PD-L1 IHC 28-8 pharmDx, example cases of various PD-L1 expression levels are provided for reference. These example cases and in-depth recommendations for the interpretation of gastric, GEJ, and esophageal adenocarcinoma specimens can help individual labs achieve reproducible and reliable results.

Note: This assay is intended for patients with tumor types containing at least 50% adenocarcinoma component.

Acknowledgment

Assay Interpretation

The clinical interpretation of any staining, or the absence of staining, must be complemented by the evaluation of proper controls. An 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 30.

Photomicrographs

Photomicrographs included in this interpretation manual are adenocarcinoma unless otherwise indicated. Squamous cell carcinoma (SQCC) specimens are included for gastroesophageal junction carcinoma and esophageal carcinoma as representations of SQCC components that can be seen within adenocarcinoma specimens. SQCC specimens are not part of the test labeling.

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

1. Tissue samples supplied by BioIVT Asterand®.
2. The data and gastroesophageal tissue used in this project were provided by Sapien Biosciences Pvt. Ltd. with appropriate ethics approval and through Trans-Hit Biomarkers Inc.
3. The data and gastroesophageal tissue used in this project were provided by GLAS with appropriate ethics approval and through Trans-Hit Biomarkers Inc.
4. The data and gastroesophageal tissue used in this project were provided by Nottingham University Hospitals NHS Trust with appropriate ethics approval and through Trans-Hit Biomarkers Inc.
5. Biological materials were provided by the Ontario Tumour Bank, which is supported by the Ontario Institute for Cancer Research through funding provided by the Government of Ontario.
6. The data and gastroesophageal tissue used in this project were provided by National BioService LLC with appropriate ethics approval and through Trans-Hit Biomarkers Inc.
7. Samples and tissues supplied by Conversant Biologics.

PD-L1 Overview

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

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

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

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

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

Anti-PD-1 therapy works by blocking the PD-1/PD-L1 interaction between tumor cells and activated T-cells, helping to prevent immunosuppression, thereby enabling cytotoxic T-cells to actively remove tumor cells.

PD-L1 IHC 28-8 pharmDx Detects PD-L1 in Gastric, GEJ, and Esophageal Adenocarcinoma

PD-L1 upregulation in gastric, GEJ, and esophageal adenocarcinoma is a biomarker for response to anti-PD-1 therapy. PD-L1 IHC 28-8 pharmDx was the only PD-L1 assay used in the OPDIVO (nivolumab) clinical trial (CheckMate-649) to evaluate the relationship between PD-L1 expression and clinical efficacy.

The Role of the PD-1/PD-L1 Pathway in Cancer

Limiting damage to healthy tissue

Inactivation of T-cells limits damage to healthy tissue.

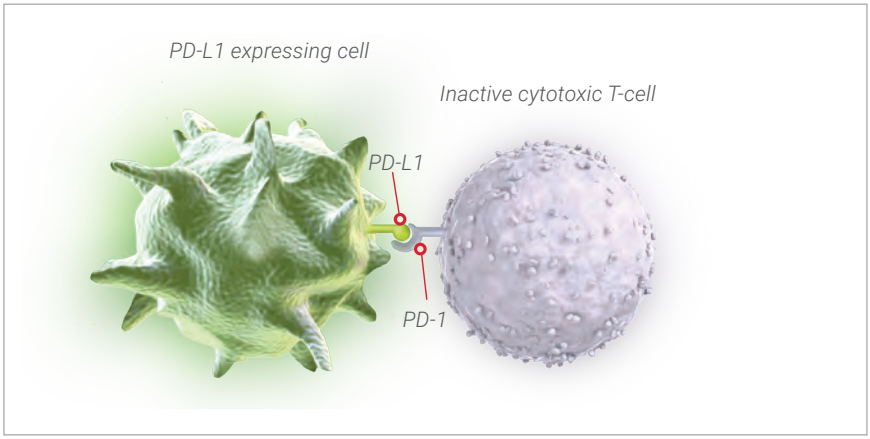


Figure a.

The tumor escapes detection

Inactivation of T-cells reduces tumor cell killing.

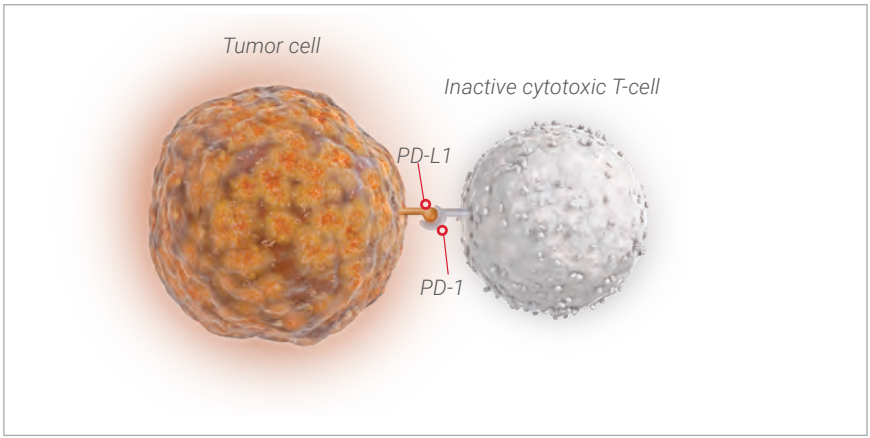


Figure b.

Immuno-oncology therapies harness the immune response to fight tumors

Blocking PD-L1 enables cytotoxic T-cells to actively remove tumor cells.

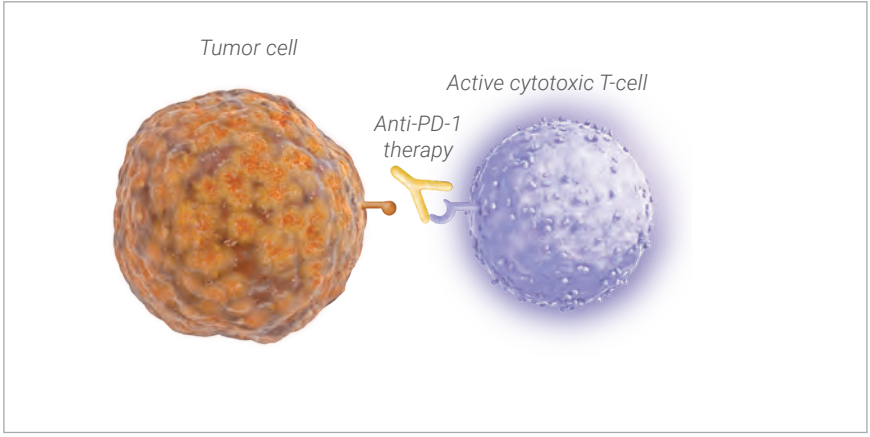


Figure c.

Study Data for PD-L1 IHC 28-8 pharmDx in Gastric, GEJ, and Esophageal Adenocarcinoma

CheckMate-649 results highlight overall survival (OS) benefit from OPDIVO (nivolumab) in combination with fluoropyrimidine and platinum-based chemotherapy for gastric, GEJ, and esophageal adenocarcinoma patients whose tumors express PD-L1 with a Combined Positive Score (CPS) ≥ 5.^

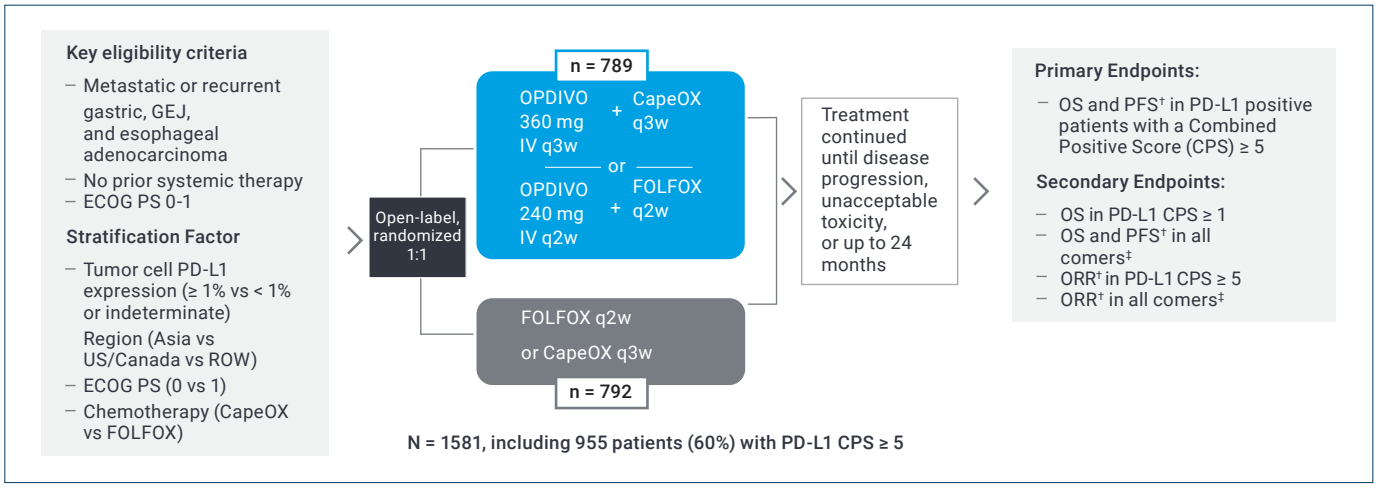
- CheckMate-649 investigated the validity of PD-L1 IHC 28-8 pharmDx for the assessment of PD-L1 status in gastric, GEJ, and esophageal adenocarcinoma patients treated with OPDIVO (nivolumab) in combination with fluoropyrimidine and platinum-based chemotherapy.
- CheckMate-649 is a phase 3, randomized, multi-center, open-label study in patients who were previously untreated with HER2-negative, advanced or metastatic gastric cancer whose tumors express PD-L1 with a Combined Positive Score (CPS) ≥ 5.

In randomized phase 3 study OPDIVO (nivolumab) + fluoropyrimidine and platinum-based chemotherapy was assessed in patients with unresectable advanced or recurrent gastric, GEJ, and esophageal adenocarcinoma

CheckMate-649** Study Design (n=1,581)

A randomized, phase 3 study of 1L I-O + chemotherapy* in HER2-negative + advanced gastric, GEJ, and esophageal adenocarcinoma

The prevalence of PD-L1 CPS ≥ 5 in CheckMate-649 was 60%



Note:
- The trial excluded patients who were known HER2+ or had untreated CNS metastases
- Tumor specimens were evaluated prospectively using the PD-L1 IHC 28-8 pharmDx assay at a central laboratory
- The efficacy analysis in patients with PD-L1 CPS ≥ 5 included 473 patients in the OPDIVO + FOLFOX or CapeOX arm and 482 patients in the FOLFOX or CapeOX arm
- In patients in whom chemotherapy was discontinued, OPDIVO monotherapy was allowed to be given at 240 mg q2w, 360 mg q3w, or 480 mg q4w up to 2 years after treatment initiation
- Median follow-up time was 12.1 months

^Moehler ESMO 2020 presentation.

**FOLFOX or CapeOX. †Assessed using blinded independent central review (BICR). ECOG PS=Eastern Cooperative Oncology Group Performance Status; IHC=immunohistochemistry; IV=intravenous; ORR=overall response rate; PFS=Progression-free survival; OS=overall survival; EAC=Esophageal adenocarcinoma*

***Janjigian Y. Y., Shitara K., et al. First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastroesophageal junction, and esophageal adenocarcinoma (CheckMate-649): a randomised, open-label, phase 3 trial. The Lancet. 2021*

The Clinical Value of PD-L1 IHC 28-8 pharmDx Expression in Gastric, GEJ, and Esophageal Adenocarcinoma

– As per CheckMate-649 study results, in patients whose tumors express PD-L1 with a CPS ≥ 5 the **Median Duration of Overall Survival** was **14.4 months** with OPDIVO (nivolumab) + chemotherapy FOLFOX or CapeOX and **11.1 months** with chemotherapy group alone.

Efficacy outcomes in patients whose tumors express PD-L1 with a CPS ≥ 5:
Overall survival with OPDIVO (nivolumab) + chemotherapy FOLFOX or CapeOX and chemotherapy group alone.

Table 1. Efficacy results in patients with PD-L1 CPS ≥ 5 (CA209649)

	nivolumab + chemotherapy (n = 473)	**chemotherapy (n = 482)**
	Minimum follow up 19.4 months[a]	
Overall Survival		
Events	344 (73%)	397 (82%)
Hazard ratio (95% CI)[b]	0.69 (0.60, 0.81)	
Median (95% CI) (months)[c]	14.4 (13.1, 16.3)	11.1 (10.0, 12.1)
Rate (95% CI) at 12 months	57.3 (52.6, 61.6)	46.4 (41.8, 50.8)
Progression-free survival[d]		
Events	342 (72.3%)	366 (75.9%)
Hazard ratio (95% CI)[b]	0.68 (0.59, 0.79)	
Median (95% CI) (months)[c]	8.31 (7.03, 9.26)	6.05 (5.55, 6.90)
Rate (95% CI) at 12 months	36.3 (31.7, 41.0)	21.9 (17.8, 26.1)
Objective response rate, n[d, e]	**227/378 (60%)**	**176/390 (45%)**
(95% CI)	(54.9, 65.0)	(40.1, 50.2)
Complete response	12.2%	6.7%
Partial response	47.9%	38.5%
Duration of response[d, e]		
Median (95% CI) (months)[c]	9.69 (8.25, 12.22)	6.97 (5.62, 7.85)

[a] Descriptive analysis based on data cut-off: 04-Jan-2021
[b] Based on stratified long Cox proportional hazard model
[c] Kaplan-Meier estimate
[d] Confirmed by BICR
[e] Based on patients with measurable disease at baseline

PD-L1 IHC 28-8 pharmDx Overview

Code SK005

PD-L1 IHC 28-8 pharmDx contains optimized reagents and the protocol required to complete an IHC staining procedure of formalin-fixed, paraffin-embedded (FFPE) specimens using Autostainer Link 48 and PT Link Pre-treatment module.

Following incubation with the primary monoclonal antibody to PD-L1 or the Negative Control Reagent (NCR), specimens are incubated with a linker antibody specific to the host species of the primary antibody, then incubated with a ready-to-use visualization reagent consisting of secondary antibody molecules and horseradish peroxidase molecules coupled to a dextran polymer backbone. The enzymatic conversion of the subsequently added chromogen results in the precipitation of a visible reaction product at the site of the antigen. The color of the chromogenic reaction is modified by a chromogen enhancement reagent. The specimen may then be counterstained and coverslipped. Results are interpreted using a light microscope. Control Slides containing two FFPE human cell lines are provided to validate staining runs.

PD-L1 IHC 28-8 pharmDx staining procedure

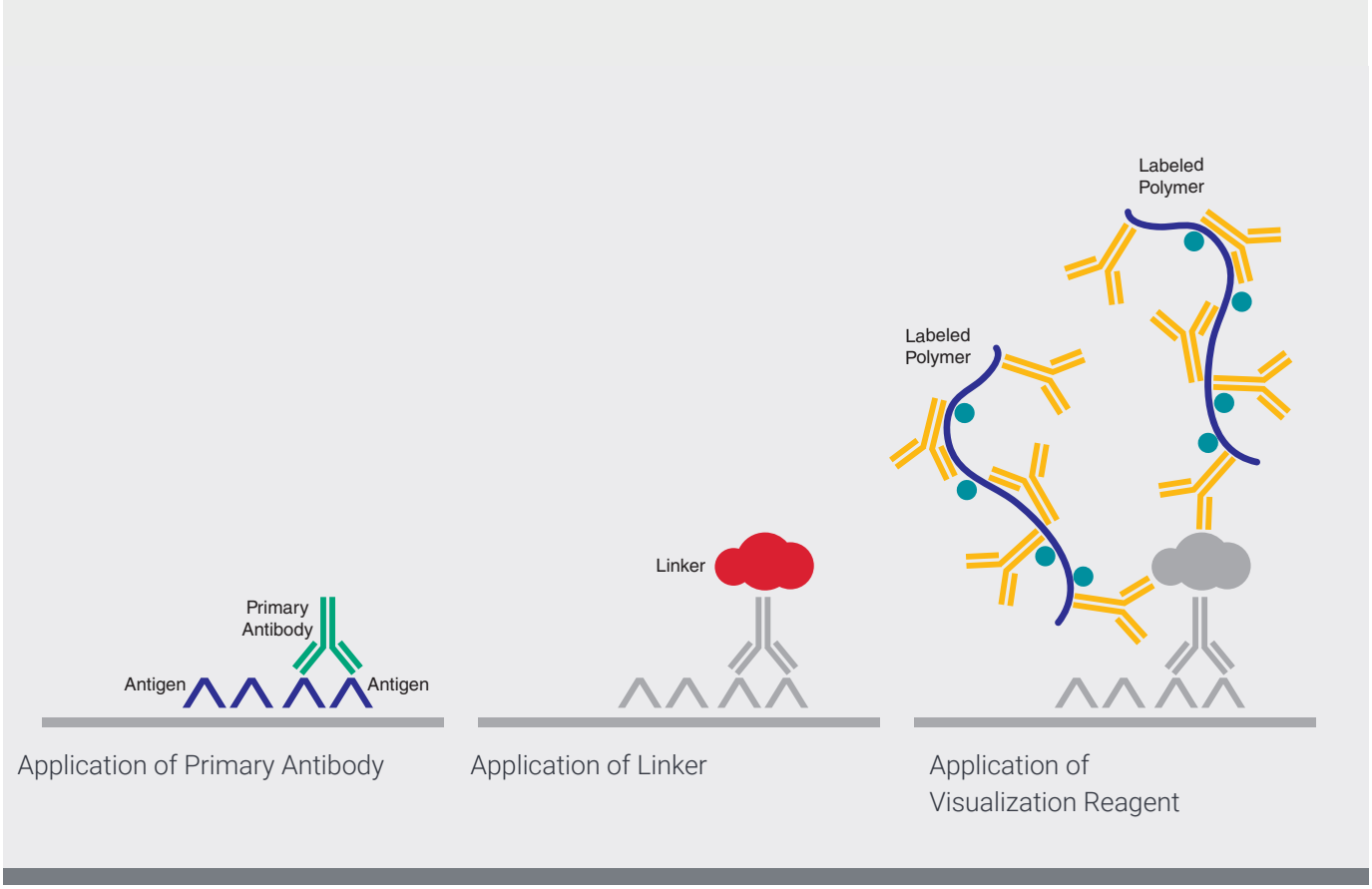


Figure 1a. PD-L1 IHC 28-8 pharmDx staining procedure.

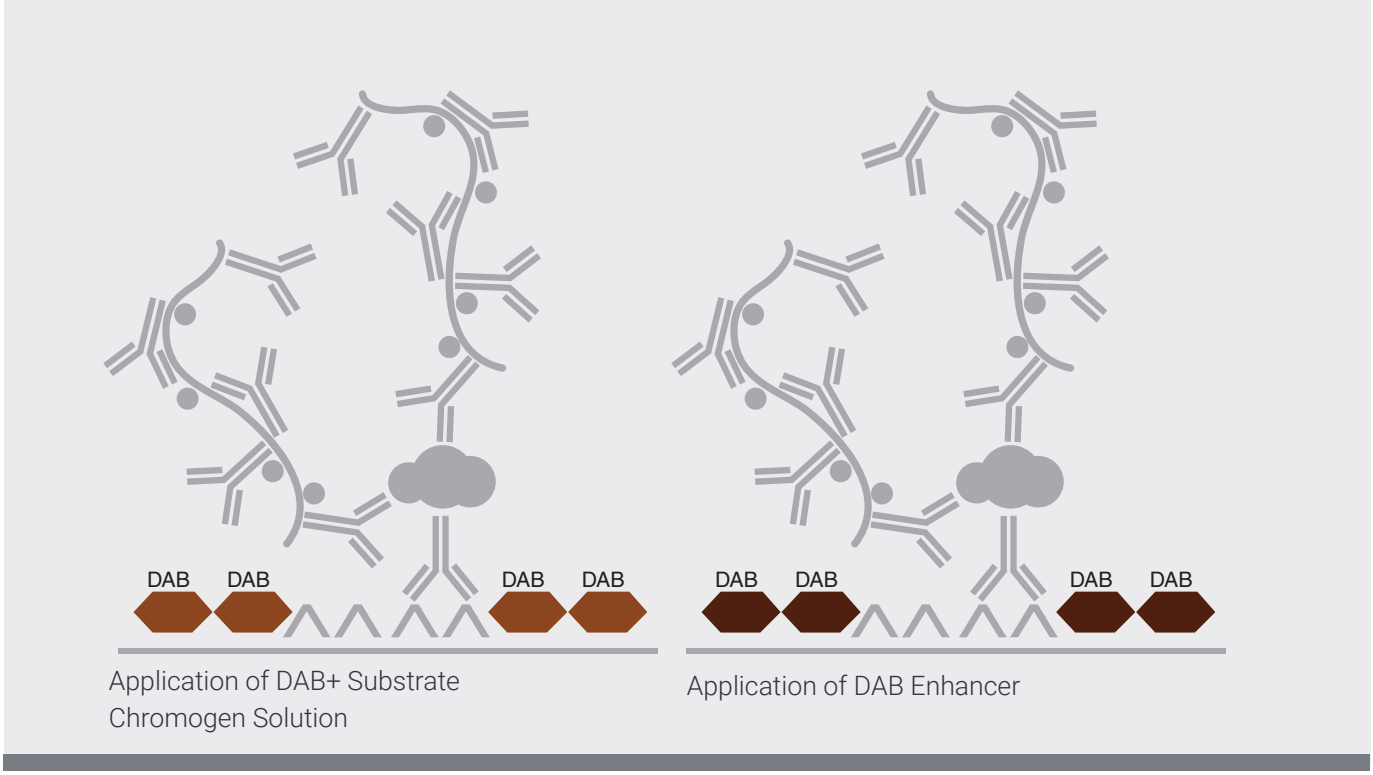


Figure 1b. PD-L1 IHC 28-8 pharmDx staining procedure.

Figure 2. PD-L1 IHC 28-8 pharmDx staining components.

All PD-L1 IHC 28-8 pharmDx reagents are to be used on the Autostainer Link 48. All reagents must be used as indicated in the IFU in order for the test to perform as specified.

PD-L1 IHC 28-8 pharmDx contains reagents to perform 50 tests in up to 15 individual runs (see Figure 2).

- EnVision FLEX Target Retrieval Solution, Low pH, 50x
- Peroxidase-Blocking Reagent
- Primary Antibody: Monoclonal Rabbit Anti-PD-L1, Clone 28-8
- Negative Control Reagent
- PD-L1 IHC 28-8 pharmDx Anti-Rabbit LINKER
- Visualization Reagent-HRP
- DAB+ Substrate Buffer
- DAB+ Chromogen
- DAB Enhancer
- PD-L1 IHC 28-8 pharmDx Control Slides

EnVision FLEX Wash Buffer (20x) (Code K8007), EnVision FLEX Hematoxylin (Code K8008), are required but not included in the kit. Refer to the IFU for required materials and equipment.

Technical Considerations for Optimal Performance of PD-L1 IHC 28-8 pharmDx

Optimal staining performance is achieved by adhering to the PD-L1 IHC 28-8 pharmDx protocol. Technical problems relating to the performance of PD-L1 IHC 28-8 pharmDx may arise in two areas; those involving specimen collection and specimen preparation prior to performing the test, as well as problems involving the actual performance of the test itself. Technical problems related to the performance of the test generally are related to procedural deviations and can be controlled and minimized through training and a thorough understanding of the product instructions by the user.

Specimen Collection and Preparation

Specimens must be handled in a way that preserves the tissue for immunohistochemical staining. Tissue should be stained and interpreted as close to the time of biopsy as possible. Use the recommended methods of tissue processing for all specimens.

Tissue Processing

FFPE tissues are suitable for use. An ischemia time from excision to formalin fixation start time of < 30 minutes, followed by immersion in 10% neutral buffered formalin (NBF) for 24–48 hours is recommended. Specimens should be blocked into a thickness of 3 mm or 4 mm, fixed in 10% NBF, and dehydrated and cleared in a series of alcohols and xylene, followed by infiltration with melted paraffin. The paraffin temperature should not exceed 60 °C. The use of PD-L1 IHC 28-8 pharmDx on decalcified tissues has not been validated and is not recommended.

Cut tissue specimens into sections of 4–5 µm. After sectioning, mount tissues on FLEX IHC microscope slides (Code K8020), or Superfrost Plus charged slides and then place in a 58 ± 2 °C oven for 1 hour.

Positive and Negative Control Tissues (Lab-supplied)

Differences in processing and embedding in the user's laboratory may produce significant variability in results. Include positive and negative control tissue in each staining run, in addition to the PD-L1 IHC 28-8 pharmDx Control Slide.

Control tissue must represent one of the approved tumor indications for PD-L1 IHC 28-8 pharmDx as listed in the Intended Use of the IFU. Fix, process, and embed the control tissue in the same manner. Control tissues processed differently from the patient specimen validate reagent performance only and do not verify tissue preparation.
The ideal positive control tissue provides a complete dynamic representation of weak-to-moderate staining when stained with PD-L1. The ideal negative control tissue should demonstrate no staining on tumor cells and immune cells. However, because the prevalence of PD-L1 expression on immune cells is high, a few staining immune cells are acceptable.

PD-L1 IHC 28-8 pharmDx Staining Procedure

The PD-L1 IHC 28-8 pharmDx reagents and instructions have been designed for optimal performance. Further dilution of the reagents, alteration of incubation times, temperatures, or instruments may give erroneous results.

Reagent Storage

Store all components of PD-L1 IHC 28-8 pharmDx, including Control Slides, in the dark at 2–8 °C when not in use on Autostainer Link 48. Do not use after the expiration date printed on the outside package.

Reagent Preparation

Equilibrate all components to room temperature (20–25 °C) prior to immunostaining.

EnVision FLEX Target Retrieval Solution, Low pH (50x)

Prepare a sufficient quantity of 1x Target Retrieval Solution, Low pH by diluting Target Retrieval Solution, Low pH (50x) 1:50 using reagent-quality water; the pH of 1x Target Retrieval Solution should be 6.1 ± 0.2. One 30 mL bottle of Target Retrieval Solution, Low pH (50x), diluted 1:50 will provide 1.5 L of 1x reagent, sufficient to fill one PT Link tank, which will treat up to 24 slides per use. Discard 1x Target Retrieval Solution after three uses and no longer than 5 days after dilution. Note the FLEX Target Retrieval Solution, low pH (50x) is a red-colored solution.

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

EnVision FLEX Wash Buffer (20x)

Prepare a sufficient quantity of 1x EnVision FLEX Wash Buffer for the wash steps by diluting Wash Buffer (20x) 1:20 using distilled or deionized water and mix thoroughly. Store unused 1x solution at 2–8 °C for no more than one month. Discard buffer if cloudy in appearance. Refer to the User Guide for your Autostainer Link 48 for further information. EnVision FLEX Wash Buffer (20x) is available as Code K8007.

DAB+ Substrate-Chromogen Solution

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

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

Control Slides

Each slide contains sections of two pelleted, FFPE cell lines: NCI-H226** with positive PD-L1 protein expression (originating from human lung squamous cell carcinoma with positive PD-L1 protein expression) and MCF-7 with negative PD-L1 protein expression (originating from human breast adenocarcinoma with negative PD-L1 protein expression).

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

Staining Protocol

Program slides by selecting PD-L1 IHC 28-8 pharmDx staining protocol from the options in the DakoLink drop-down menu. All of the required steps and incubation times for staining are preprogrammed in the DakoLink software. Print and attach slide labels to each slide.

Deparaffinization, Rehydration, and Target Retrieval

Use PT Link, Code PT100/PT101/PT200, to perform the Deparaffinization, Rehydration, and Target Retrieval 3-in-1 procedure.

- Set Pre-heat and Cool to 65 °C, and set Heat to 97 °C for 20 minutes.
- Fill PT Link tanks with 1.5 L per tank of EnVision FLEX Target Retrieval Solution, Low pH, working solution to cover the tissue sections.
- Pre-heat the Target Retrieval Solution, Low pH to 65 °C.
- Immerse Autostainer racks containing mounted, FFPE tissue sections into the pre-heated Target Retrieval Solution, Low pH in PT Link tank. Incubate for 20 minutes at 97 °C.
- When Target Retrieval incubation has been completed, and the temperature has cooled to 65 °C, remove each Autostainer slide rack with slides from the PT Link tank and immediately place the slides into a tank (e.g., PT Link Rinse Station, Code PT109) containing room temperature EnVision FLEX Wash Buffer working solution.
- Leave Autostainer rack with slides in room temperature EnVision FLEX Wash Buffer for 5 minutes.

Staining and Counterstaining

- Place the Autostainer rack with slides on the Autostainer Link 48. Ensure slides remain wet with buffer while loading and prior to initiating the run. Dried tissue sections may display increased non-specific staining.
- The instrument performs the staining and counterstaining procedures by applying the appropriate reagent, monitoring the incubation time, and rinsing slides between reagents. Counterstaining using EnVision FLEX Hematoxylin (Code K8008), for 7 minutes, is included in the staining protocol. Do not allow slides to dry prior to mounting.

Mounting

Use non-aqueous permanent mounting media. To minimize fading, store slides in the dark at room temperature (20–25 °C).

PD-L1 IHC 28-8 pharmDx Technical Checklist

Customer Name/Institution: _____

Name and Title: _____

Autostainer Link 48 Serial Number: _____ Software Version: _____

		Yes	No
1.	Regular preventive maintenance is performed on the Autostainer Link 48 and PT Link?	☐	☐
2.	PD-L1 IHC 28-8 pharmDx is used before the expiration date printed on the outside of the box?	☐	☐
3.	All PD-L1 IHC 28-8 pharmDx components, including Control Slides, are stored in the dark at 2–8 °C?	☐	☐
4.	All PD-L1 IHC 28-8 pharmDx components, including Control Slides, are equilibrated to room temperature (20–25 °C) prior to immunostaining?	☐	☐
5.	Appropriate positive and negative control tissues are identified?	☐	☐
6.	Tissues are fixed in neutral buffered formalin?	☐	☐
7.	Tissues are infiltrated with melted paraffin, at or below 60 °C?	☐	☐
8.	Tissue sections of 4–5 µm are mounted on FLEX IHC Microscope Slides, or Superfrost Plus charged slides?	☐	☐
9.	Gastric, GEJ, and esophageal adenocarcinoma specimens are stained within 4 months of sectioning when stored in the dark at 2–8 °C or at room temperature up to 25 °C?	☐	☐
10.	EnVision FLEX Target Retrieval Solution, Low pH is prepared properly?	☐	☐
11.	EnVision FLEX Wash Buffer is prepared properly?	☐	☐
12.	DAB+ Substrate-Chromogen Solution is prepared properly?	☐	☐
13.	The Deparaffinization, Rehydration, and Target Retrieval 3-in-1 procedure is followed, using PT Link?	☐	☐
14.	Slides remain wet with buffer while loading and prior to initiating run on Autostainer Link 48?	☐	☐
15.	The PD-L1 IHC 28-8 pharmDx protocol is selected on Autostainer Link 48?	☐	☐
16.	Slides are counterstained with EnVision FLEX Hematoxylin?	☐	☐
17.	Do you have all the necessary equipment to perform the PD-L1 IHC 28-8 pharmDx test according to the protocol? If not, specify what is missing in the comments below.	☐	☐

If you answered "No" to any of the above, consult with your local Dako Technical Support Representative for assistance.

Additional Observations or Comments:

_____

_____

_____

Recommendations for Interpretation of PD-L1 IHC 28-8 pharmDx in Gastric, GEJ, and Esophageal Adenocarcinoma

PD-L1 IHC 28-8 pharmDx evaluation must be performed by a pathologist using a bright field microscope. Before examining the patient specimen for PD-L1 staining, it is important to examine the hematoxylin and eosin (H&E) and controls first. Examine a serial section of the patient specimen stained with H&E for histology and preservation quality. Then, examine the PD-L1 IHC 28-8 pharmDx Control Slide, the positive and negative control tissue slides, and the patient specimen slide stained with the Negative Control Reagent (NCR) to assess reagent performance. Lastly, examine the patient specimen stained with Primary Antibody to assess the staining of viable tumor and mononuclear inflammatory cells.

PD-L1 staining is defined as complete circumferential or partial linear plasma membrane staining of tumor cells and mononuclear inflammatory cells (MICs), at any intensity. Cytoplasmic staining is considered positive for MICs only, any cytoplasmic staining in tumor cells should be ignored. MICs are included in the evaluation of the CPS numerator and should be included during determination of PD-L1 positivity.

Positive control tissue slides and negative control tissue slides should be supplied by the laboratory. Only the Control Slide is provided in the PD-L1 IHC 28-8 pharmDx.

Patient Specimen Stained with H&E

An H&E stained section is required for the evaluation of histology and preservation quality. PD-L1 IHC 28-8 pharmDx and the H&E staining should be performed on a serial section from the same paraffin block of the specimen.

PD-L1 IHC 28-8 pharmDx Control Slide

Examine the PD-L1 IHC 28-8 pharmDx Control Slide to ascertain that reagents are functioning properly. Each slide contains sections of cell pellets with positive and negative PD-L1 expression, see Figure 3. If any staining of the Control Slide is not satisfactory, all results with the patient specimens should be considered invalid. Do not use the Control Slide as an aid in the interpretation of patient results.

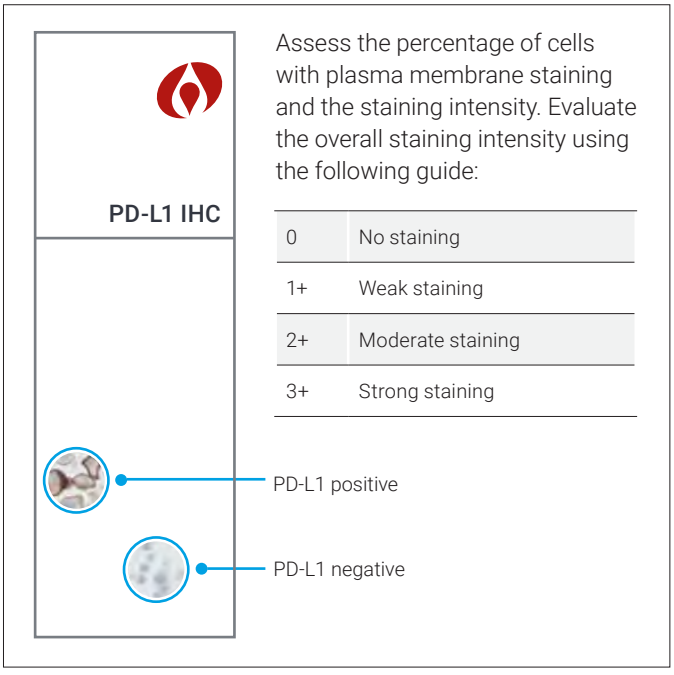


Figure 3. Each Control Slide contains sections of cell pellets with positive and negative PD-L1 expression.

For the PD-L1 positive cell pellet, the following staining is acceptable, see Figure 4.

- Plasma membrane staining of ≥ 80% of cells
- ≥ 2+ average staining intensity of cells with membrane staining
- Non-specific staining < 1+ intensity

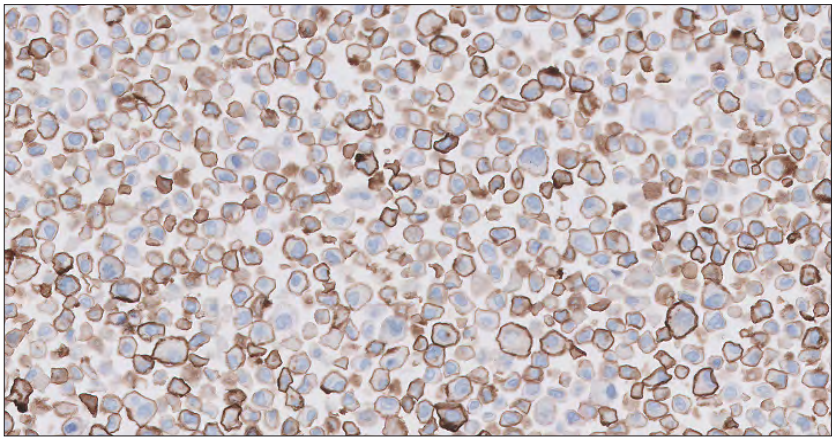


Figure 4. Acceptable Positive PD-L1 Control.

For the PD-L1 negative cell pellet, the following staining is acceptable, see Figure 5.

- No plasma membrane staining
- Any non-specific staining is of < 1+ staining intensity

Staining of a few cells in the negative pellet may occasionally be observed. The presence of 10 or less cells with distinct plasma membrane staining, and/or cytoplasmic staining with ≥ 1+ intensity within the boundaries of the cell pellet is acceptable.

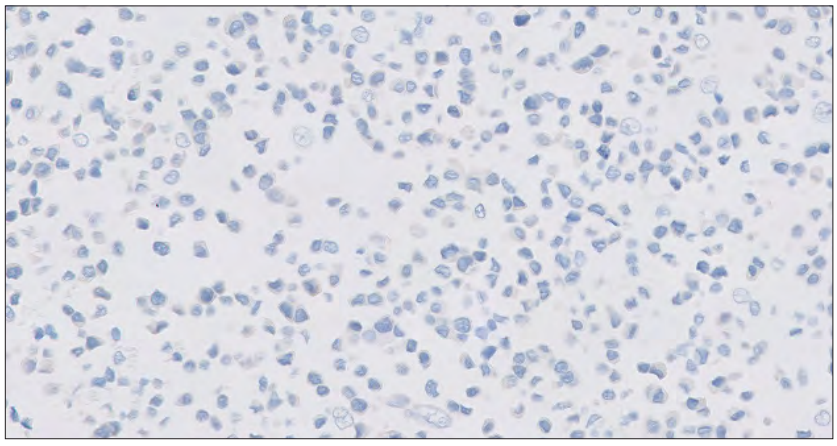


Figure 5. Acceptable Negative PD-L1 Control.

Positive Control Tissue Slides

Examine the positive control tissue slides (Primary Antibody, NCR) to ascertain if tissues are correctly prepared, and reagents are functioning properly. Any non-specific staining should be of ≤ 1+ staining intensity. Exclude necrotic or nonviable tumor cells from the evaluation. If the staining of positive control tissues is not satisfactory, all results with the patient specimens should be considered invalid. Do not use control tissue as an aid in the interpretation of patient results.

Negative Control Tissue Slides

Examine the negative control tissue slides (Primary Antibody, NCR) to confirm that there is no unintended staining. Any non-specific staining should be ≤ 1+ staining intensity. If plasma membrane staining of tumors cells occurs in the negative control tissue, all results with the patient specimens should be considered invalid. Do not use control tissue as an aid in the interpretation of patient results.

Patient Specimen Stained with Negative Control Reagent

Examine the patient specimen stained with NCR to ascertain that reagents are functioning properly. The absence of plasma membrane staining of viable tumor cells is satisfactory and non-specific staining should be ≤ 1+ staining intensity. If any staining is not satisfactory, results with the patient specimen should be considered invalid.

The NCR indicates non-specific staining and allows better interpretation of the patient specimen stained with the Primary Antibody.

Patient Specimen Stained with Primary Antibody

Staining should be assessed within the context of any non-specific staining of the patient specimen stained with NCR. A minimum of 100 viable tumor cells should be present in the PD-L1 stained patient slide in order to perform an evaluation.

1. At 4–20x objective magnification, carefully examine the tumor areas of the entire specimen. All areas with viable tumor cells on the specimen should be evaluated. Exclude necrotic cells and cellular debris. Non-specific cytoplasmic staining, if present, should be disregarded.
2. A 20x objective magnification is required to determine the PD-L1 expression. Tumor cells are considered to be PD-L1 positive if they exhibit either partial linear or complete circumferential staining of the plasma membrane at any intensity. MICs are considered positive if they exhibit partial or complete membrane staining, and/or cytoplasmic staining, at any intensity.
3. When determining the CPS of the entire specimen, the numerator is the number of stained viable tumor cells and tumor-associated MICs (lymphocytes and macrophages^) and the denominator is the total number of viable tumor cells in the specimen. Record if the specimen has PD-L1 expression of CPS ≥ 5.

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

^ *Macrophages and histiocytes are considered the same cells*
* *Note: Even though a calculated CPS may be greater than 100, only a maximum CPS of 100 should be given*

Tips and Special Considerations

- Include the entire specimen for evaluation of PD-L1 expression
- Use higher magnifications to confirm cell types and areas absent of staining
- Be careful not to overlook weak 1+ staining, which can be missed at 4x and 10x
- Disregard non-specific staining
- Necrotic tissue may stain but should be excluded
- Granular staining must demonstrate a perceptible and convincing membrane pattern

Indeterminate Specimen

High cytoplasmic staining of tumor cells can hamper the scoring of the membrane staining. An additional cut section or section from another block of the same patient may be required for PD-L1 IHC 28-8 pharmDx evaluation.

Slide Evaluation Flowchart

The following flow of slide review is recommended when conducting interpretation of PD-L1 IHC 28-8 pharmDx. Refer to the detailed description on pages 14–17.

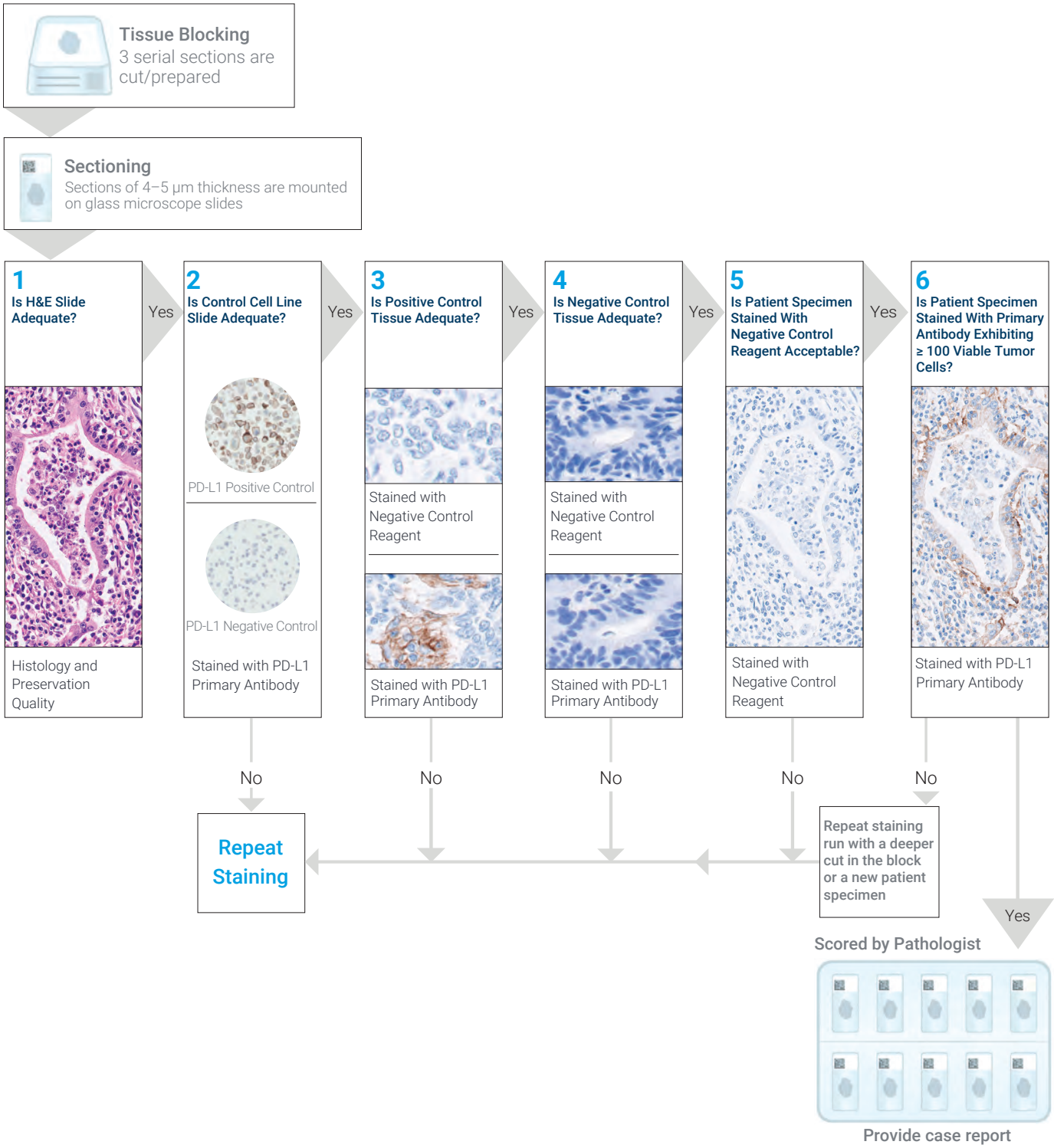


Figure 6. Slide evaluation procedure steps.

Combined Positive Score

Definition of Combined Positive Score (CPS)

For patients being considered for first-line treatment, PD-L1 expression in gastric, GEJ, and esophageal adenocarcinoma is determined using the Combined Positive Score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages^) divided by the total number of viable tumor cells, multiplied by 100. Although the result of the calculation can exceed 100, the maximum score is defined as CPS 100.

CPS is defined accordingly:

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

^ *Macrophages and histiocytes are considered the same cells*
* *Note: Even though a calculated CPS may be greater than 100, only a maximum CPS of 100 should be given.*

Table 2. CPS Numerator Inclusion/Exclusion Criteria

Tissue Elements	Included in the Numerator	Excluded from the Numerator
Tumor Cells	Convincing partial linear or complete circumferential membrane staining (at any intensity) of viable invasive and metastatic gastric, GEJ, and esophageal adenocarcinoma	– Non-staining tumor cells – Tumor cells with only cytoplasmic staining – Dysplasia – In situ 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‡ Only MICs within 20x field and directly associated with the response to the tumor are scored	– Non-staining MICs – MICs (including lymphoid aggregates) not associated with the tumor – MICs associated with in situ carcinoma – MICs associated with benign structures – Neutrophils, eosinophils, and plasma cells
Other Cells	Not included	– Normal cells – Stromal cells (including fibroblasts) – Necrotic cells and/or cellular debris

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

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

‡ *Macrophages and histiocytes are considered the same cells*

Table 3. CPS Denominator Inclusion/Exclusion Criteria

Tissue Elements	Included in the Denominator	Excluded from the Denominator
Tumor Cells	All viable, invasive tumor cells and metastatic cells	– Any necrotic or non-viable tumor cells – Dysplasia – In situ carcinoma
Immune Cells	Not included	All immune cells of any type
Other Cells	Not included	– Normal cells – Stromal cells (including fibroblasts) – Necrotic cells and/or cellular debris

Determining Combined Positive Score (CPS)

- For evaluation of the immunohistochemical staining, an objective of 4–20x magnification is appropriate. Examine the entire tumor area and evaluate overall areas of PD-L1 staining and non-staining tumor cells, keeping in mind that partial membrane staining or 1+ membrane staining may be difficult to see at low magnifications. Ensure there are at least 100 viable tumor cells in the sample
  - A minimum of 100 viable tumor cells must be present in the PD-L1 stained slide (biopsy or resection) 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. Repeat staining with a deeper cut in the block or a new patient specimen should be done in consultation with a qualified pathologist
- **For determination of PD-L1 expression, an objective of 20x magnification is required. Evaluate PD-L1 expression and calculate CPS:**
  - Determine the total number of viable tumor cells, both PD-L1 staining and non-staining (CPS denominator)
  - Determine the number of PD-L1 staining cells, tumor cells, lymphocytes, and macrophages (CPS numerator)
  - See Tables 2 and 3 on pages 24 and 25 respectively for additional CPS inclusion/exclusion criteria
  - Calculate CPS

PD-L1 IHC 28-8 pharmDx Suggested Scoring Methods for Calculating Combined Positive Score

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 CPS of various staining patterns.

Example 1: Calculation of Combined Positive Score based on a small PD-L1 staining area

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

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

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

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

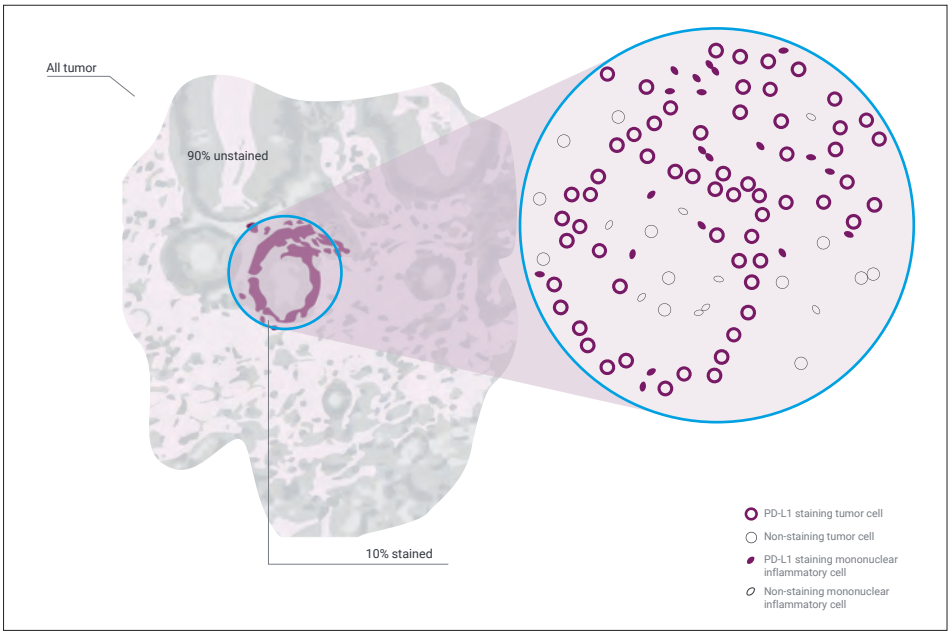


Figure 7. Example of tumor with a small PD-L1 staining area.

Calculate the CPS of the entire tumor area:

Assessment:

CPS of the area with staining:

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

^ *Tumor cells, lymphocytes, macrophages*

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

Clinical Interpretation: CPS ≥ 5

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 CPS for each region.

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

Figure 8. Example of a specimen with a heterogeneous PD-L1 staining area.

Calculate the CPS of the entire tumor area:

Assessment:

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

** Tumor cells, lymphocytes, macrophages*

(80 + 30 + 50 + 100) / 4 ≈ CPS 65

Clinical Interpretation: CPS ≥ 5

Example 3: Calculation of Combined Positive Score for a near cut-off specimen

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

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

Assessment: Tumor specimen has perceptible and convincing staining. There are 10 PD-L1 staining cells (tumor cells, lymphocytes, macrophages). There are 200 viable tumor cells present in the entire specimen.

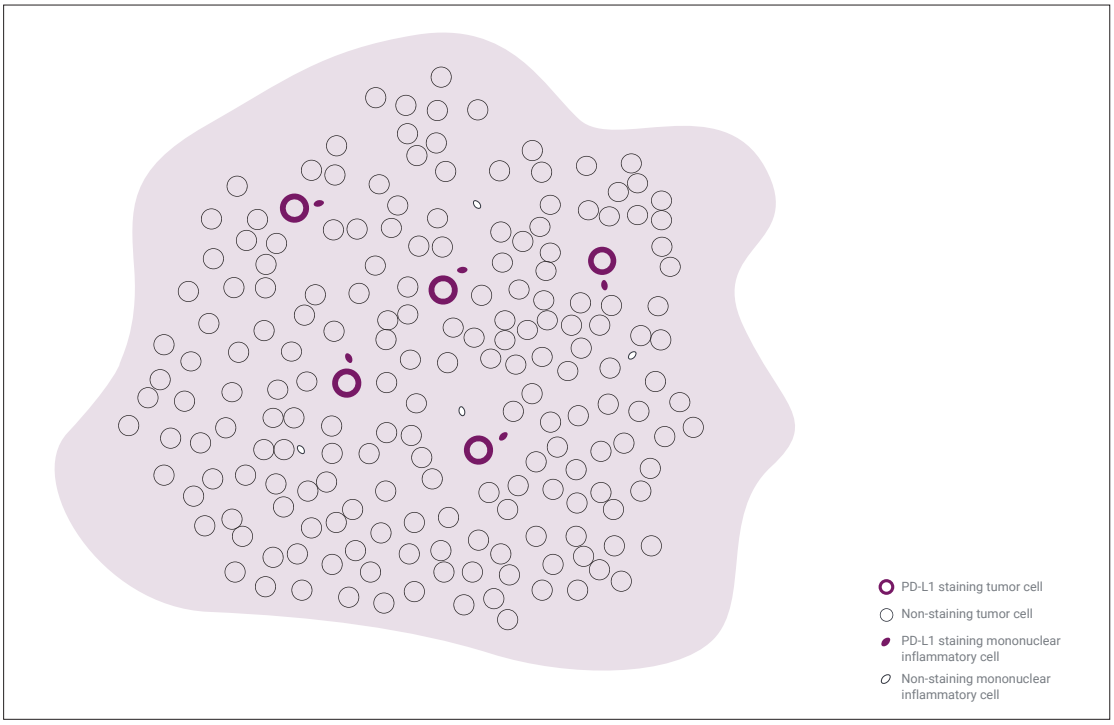


Figure 9. Example of a near cut-off specimen.

Calculate the CPS of the entire tumor area:

Assessment:

CPS of area with staining:

$$\text{CPS} = \frac{\text{\# PD-L1 staining cells}^{*}}{\text{Total \# viable tumor cells}} \times 100 = \frac{\text{10 PD-L1 staining cells}}{\text{200 tumor cells}} \times 100 = \text{CPS 5}$$

** Tumor cells, lymphocytes, macrophages*

Clinical Interpretation: CPS ≥ 5

Image Guide for Interpretation of PD-L1 IHC 28-8 pharmDx Staining in Gastric, GEJ, and Esophageal Adenocarcinoma

Agilent emphasizes that scoring of PD-L1 IHC 28-8 pharmDx must be performed in accordance with the guidelines established in the IFU, within the context of best practices and the pathologist's experience.

The CPS determines the PD-L1 IHC 28-8 pharmDx result. Scoring guidelines and reporting recommendations are presented on the following pages. See page 30 (Reporting Results form), for an example of a pathology report form for PD-L1 IHC 28-8 pharmDx. Figure 10 below demonstrates near cut-off specimens without and with PD-L1 expression.

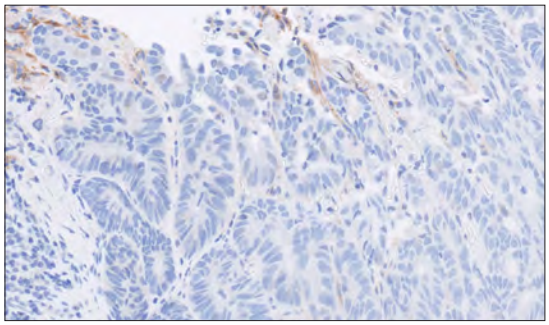
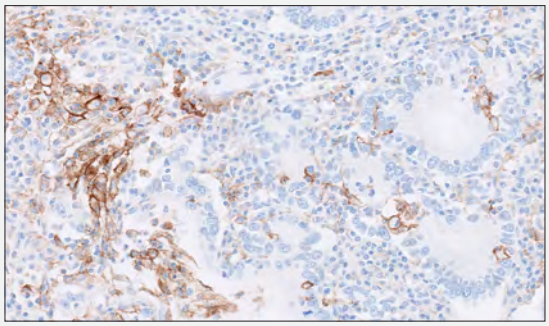
CPS	Expression Level	Image (20x magnification)
< 5	No PD-L1 Expression	
≥ 5	PD-L1 Expression	

Figure 10. Examples of near cut-off specimens.

PD-L1 IHC 28-8 pharmDx Reporting Results: Gastric, GEJ, and Esophageal Adenocarcinoma

Suggested information to include when reporting results with PD-L1 IHC 28-8 pharmDx in gastric, GEJ, and esophageal adenocarcinoma

PD-L1 IHC 28-8 pharmDx, Code SK005

Summary of Sample Tested:

Date of Run: _____ PD-L1 IHC 28-8 pharmDx Lot: _____

Staining Run Log ID: _____ Specimen ID: _____

Patient Identifier: _____

Type of Service: IHC Stain with Manual Interpretation

Other: _____

Type of Tissue: _____

Additional Tests Performed with PD-L1 IHC 28-8 pharmDx: _____

PD-L1 IHC 28-8 pharmDx Controls Results:

PD-L1 IHC 28-8 Control Cell Slides:	Pass ☐	Fail ☐
Positive Control Tissue Slides:	Pass ☐	Fail ☐
Negative Control Tissue Slides:	Pass ☐	Fail ☐
Patient Specimen, Negative Control Reagent:	Pass ☐	Fail ☐

PD-L1 Results:

PD-L1 IHC 28-8 pharmDx is indicated as an aid in identifying gastric, GEJ, and esophageal adenocarcinoma, patients for treatment with OPDIVO (nivolumab) in combination with chemotherapy fluoropyrimidine and platinum-based chemotherapy.

Viable Tumor Cells Present: ☐ ≥ 100 cells ☐ Not evaluable

☐ CPS is ≥ 5 ☐ CPS is < 5

Pathologist's comments: _____

OPDIVO is a registered trademark of Bristol-Myers Squibb Company.

Combined Positive Score Summary and Immunostaining Examples in Gastric, GEJ, and Esophageal Adenocarcinoma

Summary of CPS in PD-L1 IHC 28-8 pharmDx stained specimens

By definition, PD-L1 staining cells in gastric, GEJ, and esophageal adenocarcinoma are:

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

PD-L1 expression status in gastric, GEJ, and esophageal adenocarcinoma, is determined by CPS, which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages^) divided by the total number of viable tumor cells, multiplied by 100.

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

^ Macrophages and histiocytes are considered the same cells
** Note: Even though a calculated CPS may be greater than 100, only a maximum CPS of 100 should be given.*

This section will define and illustrate scoring inclusions and exclusions for accurate determination of the CPS. All images are adenocarcinoma unless otherwise noted.

Cells Included and Excluded from CPS

PD-L1 staining cells included in the CPS that exhibit appropriate PD-L1 staining are defined as PD-L1 staining cells. PD-L1 staining cells are included in the CPS numerator for the determination of the CPS (see Table 2 on page 24 and Table 3 on page 25 for additional CPS inclusion/exclusion criteria). All viable tumor cells should be included in the denominator. Below are common staining characteristics of PD-L1 staining cells that must be included in the CPS numerator.

Tumor cells included in CPS

Tumor cells exhibiting perceptible and convincing partial and/or complete smooth or granular linear membrane staining are considered PD-L1 staining cells. Linear membrane staining can be present at any intensity and must be perceptible and convincing at no higher than 20x magnification. Perceptible and convincing staining of tumor cells (linear membrane staining) is often heterogeneous, with various staining intensities present.

Partial and complete membrane stain

GEJ SQCC specimen stained with PD-L1 primary antibody exhibiting partial (red arrows), and complete (black arrows), linear membrane staining of tumor cells. Tumor cells exhibiting partial or complete linear membrane staining at any intensity are included in the CPS numerator.

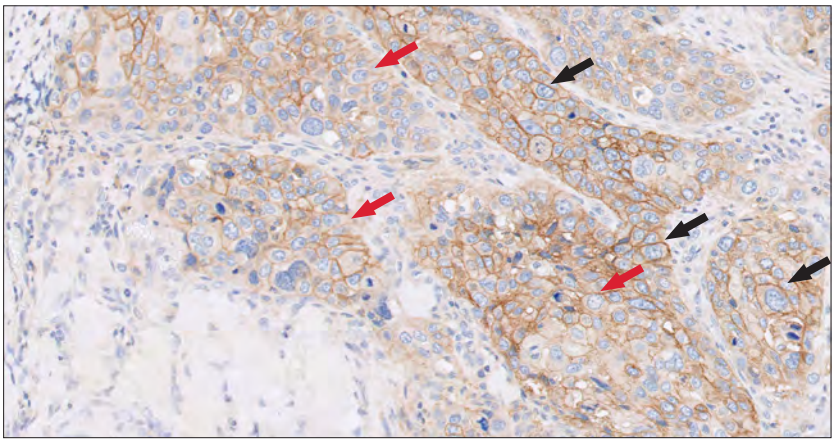


Figure 11. 20x magnification.

Linear membrane staining intensities 1-3+

EC SQCC specimen stained with PD-L1 primary antibody exhibiting mainly 1+ linear membrane staining (red arrows) of tumor cells.

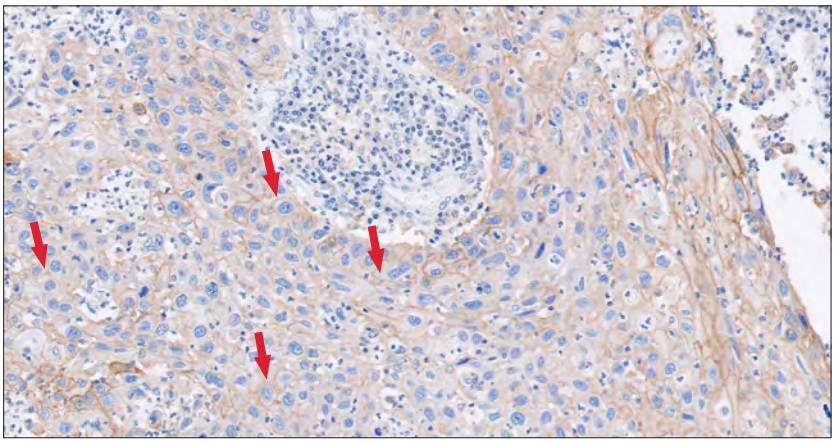


Figure 12. 20x magnification.

Linear membrane staining intensities 1-3+

EC SQCC specimen stained with PD-L1 primary antibody exhibiting mainly 2+ linear membrane staining (red arrows) of tumor cells.

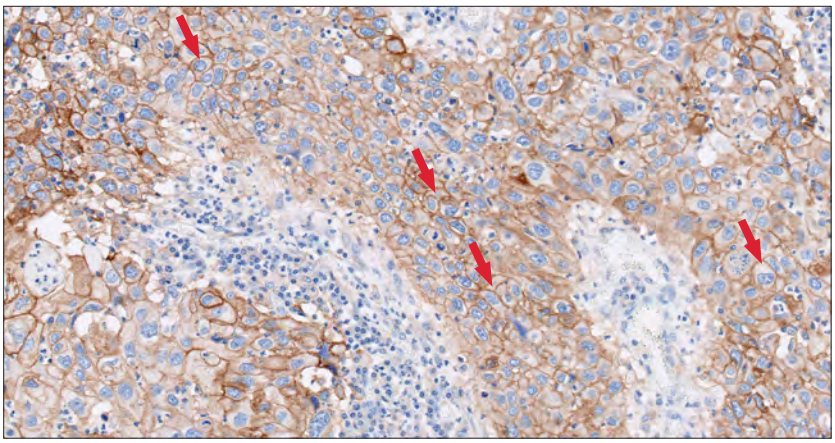


Figure 13. 20x magnification.

Linear membrane staining intensities 1-3+

GC specimen stained with PD-L1 primary antibody exhibiting mainly 3+ linear membrane staining (red arrows) of tumor cells.

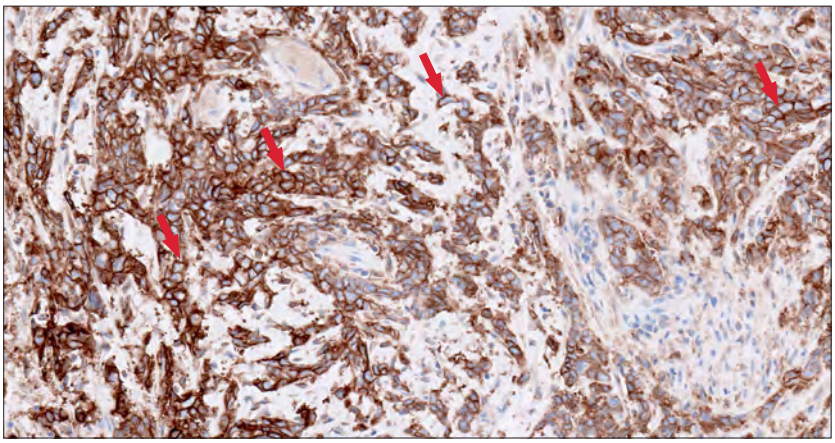


Figure 14. 20x magnification.

Key highlights

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

Linear membrane and cytoplasmic staining

EC SQCC specimen stained with PD-L1 primary antibody exhibiting linear membrane staining distinct from cytoplasmic staining (black arrows). Tumor cells with perceptible and convincing linear membrane staining distinguishable from cytoplasmic staining at 20x magnification should be included in the CPS numerator. Tumor cells exhibiting only cytoplasmic staining are excluded from the CPS numerator.

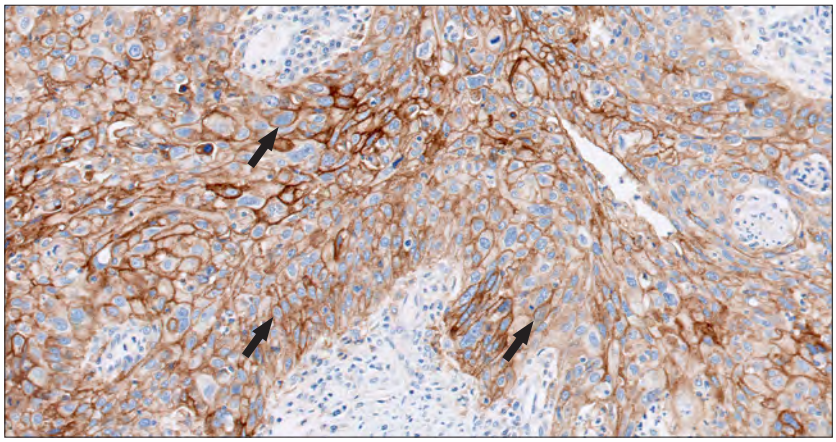


Figure 15. 20x magnification.

Linear membrane and granular staining

EC SQCC specimen stained with PD-L1 primary antibody exhibiting granular linear membrane staining pattern (black arrows).

Tumor cells can exhibit a granular staining pattern where membrane and cytoplasmic staining is indistinguishable. Only perceptible and convincing membrane staining of tumor cells at 20x magnification should be included in the CPS numerator.

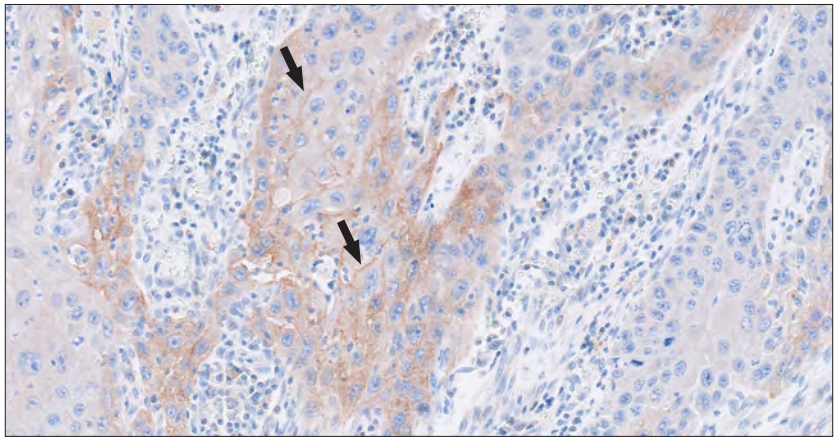


Figure 16. 20x magnification.

Key highlights

- Tumor cells exhibiting perceptible and convincing linear membrane staining in the presence of cytoplasmic or granular staining should be included in the CPS numerator
- Tumor cells with indistinguishable membrane staining in the presence of granular or cytoplasmic staining should be excluded from the CPS numerator

Multinucleate tumor cells

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

EC SQCC specimen exhibiting linear membrane staining of multinucleate tumor cells (black arrows). Some tumor cells may be multinucleate and should be counted as one cell and included in the numerator and denominator.

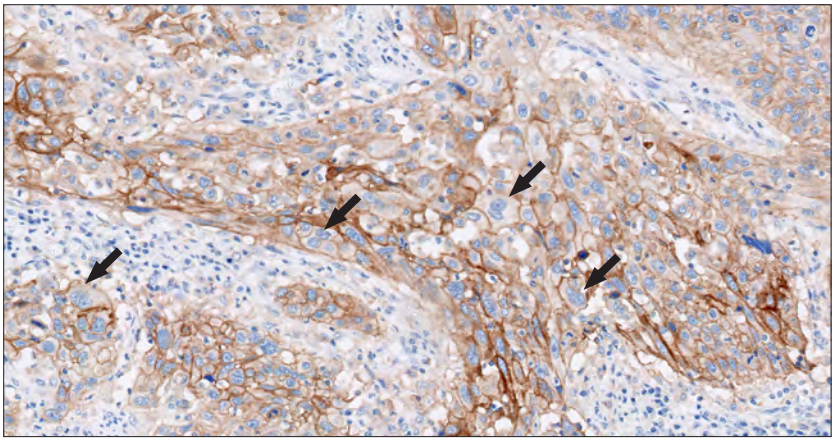


Figure 17. 20x magnification.

Signet ring cell carcinoma

Signet ring cells are commonly present in diffuse-type GC and GEJ. The tumor cell nuclei are small, crescent-shaped, and present at the periphery of the cells. Signet ring cells may be confused with macrophages which should not be included when calculating the CPS denominator.

GEJ specimen stained with PD-L1 antibody with signet ring cell morphology. Signet ring cells exhibiting any convincing partial and/or complete membrane staining at a 20x magnification (at any intensity) are considered PD-L1 staining cells and should be included in the CPS numerator.

Signet ring cells exhibiting only cytoplasmic staining should be excluded, as this is considered non-specific staining.

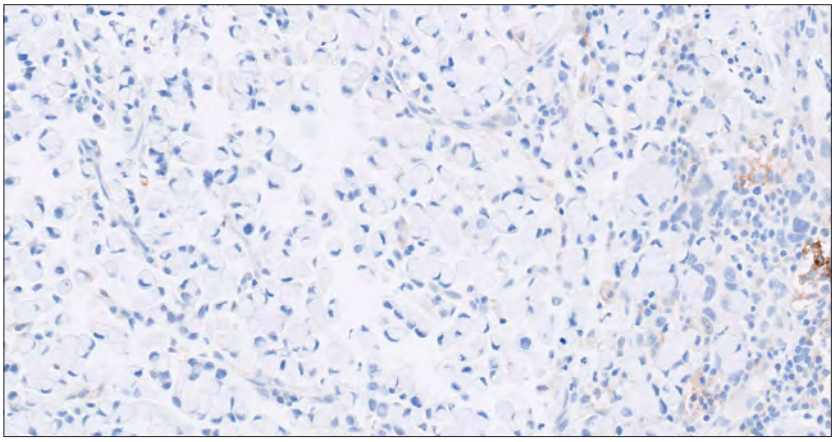


Figure 18. 20x magnification.

Key highlights

- Multinucleate tumor cells should be counted as one cell
- PD-L1 positive multinucleate tumor cells and signet ring cells should be included in the CPS numerator

Tumor cell density patterns

Gastric, GEJ, and esophageal adenocarcinoma includes different morphologies that can impact the CPS by increasing or decreasing the total number of tumor cells included in the denominator. A squamous cell component with well-differentiated, cytoplasmic rich tumor cells will commonly have fewer cells per 20x field, whereas a poorly-differentiated, basaloid pattern will commonly have a higher number of tumor cells per 20x field. The more tumor cells included in the denominator, the greater the number of PD-L1 staining tumor cells, lymphocytes, and macrophages that are needed in the numerator to bring the overall score to CPS 5 or above. As a guideline, if tumor cells are 20 µm in diameter and fill a 20x field, there would be approximately 2500 tumor cells in that field.

Low density

GEJ specimen stained with PD-L1 primary antibody exhibiting well-differentiated tumor cells with high amount of cytoplasm.

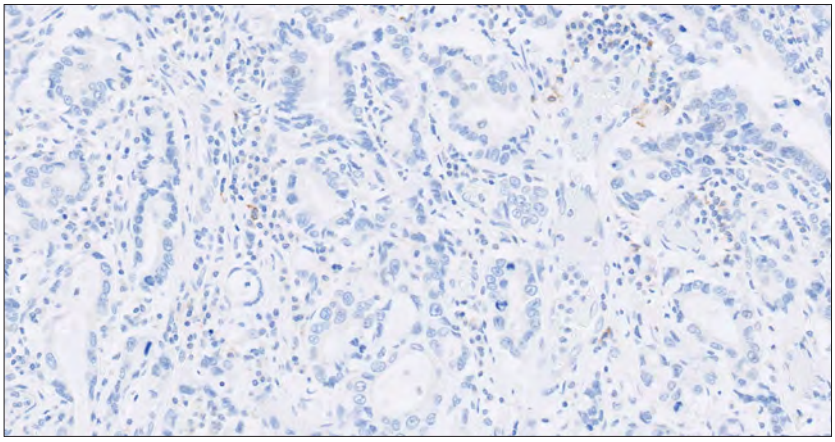


Figure 19. 20x magnification.

Moderate density

GEJ specimen exhibiting moderately differentiated tumor cells. Note the higher cell density, an indication of higher proliferative rate.

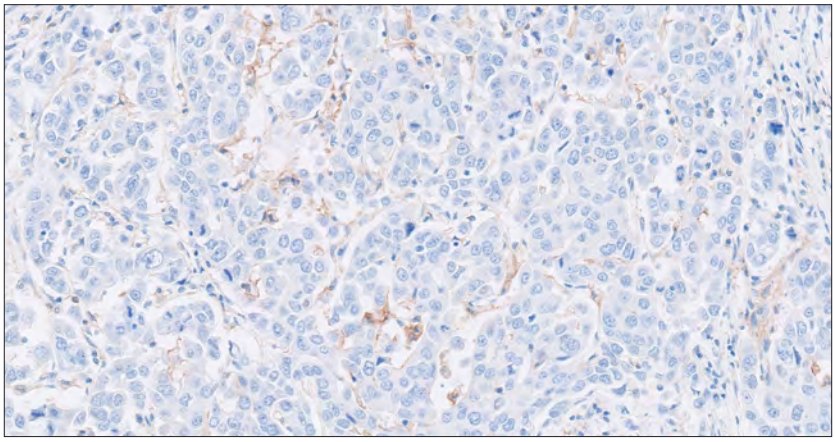


Figure 20. 20x magnification.

High density

GC specimen exhibiting poorly differentiated, basaloid tumor cells.

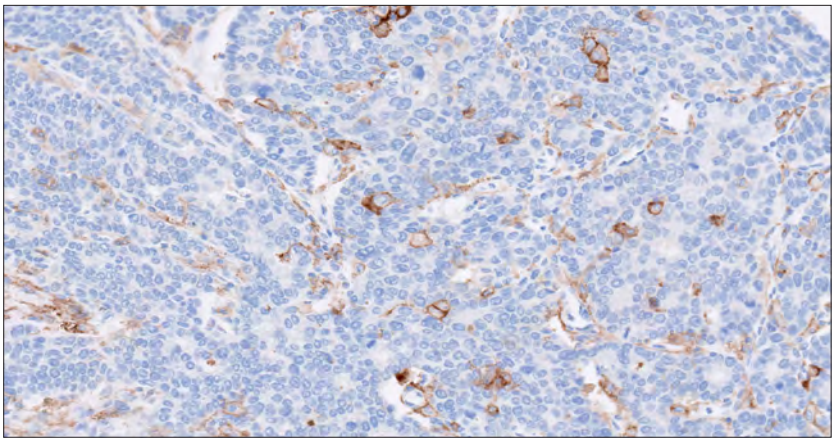


Figure 21. 20x magnification.

Key highlights

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

Immune cells included in CPS

Tumor-associated mononuclear inflammatory cells (MICs)

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

PD-L1 staining lymphocytes and macrophages

GC specimen stained with PD-L1 primary antibody exhibiting linear membrane staining and/or cytoplasmic staining of tumor-associated macrophages (black arrows), and lymphocytes (red arrows).

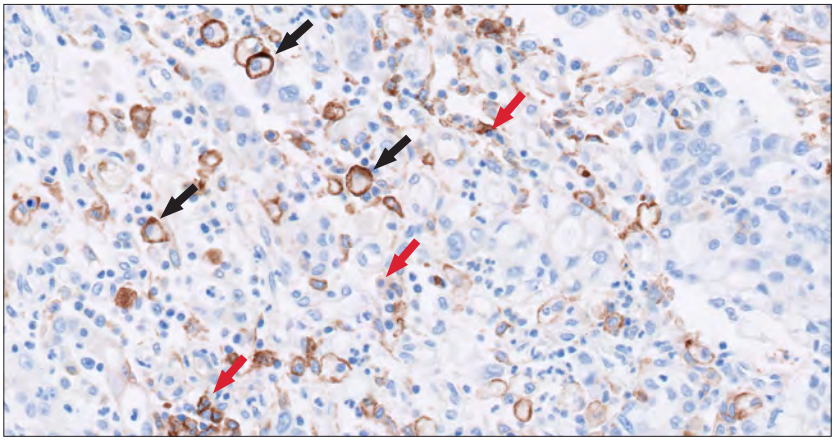


Figure 22. 20x magnification.

PD-L1 staining lymphocytes

GEJ specimen stained with PD-L1 primary antibody exhibiting linear membrane and/or cytoplasmic staining of tumor-associated lymphocytes (black arrows).

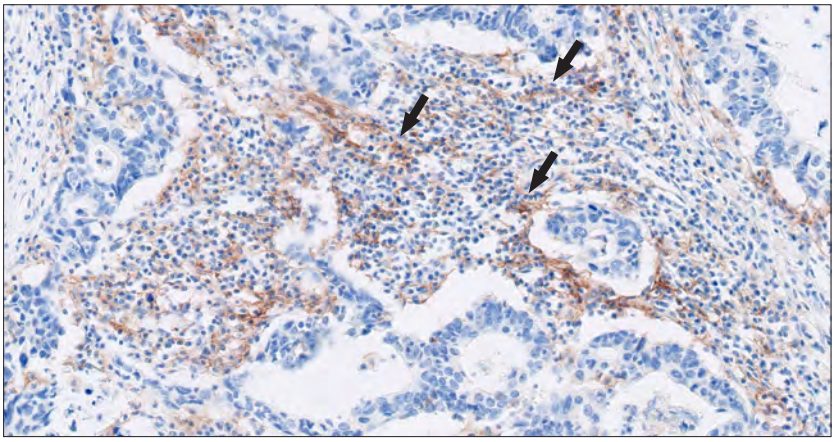


Figure 23. 20x magnification.

PD-L1 staining macrophages

GC specimen stained with PD-L1 primary antibody exhibiting linear membrane staining of tumor-associated macrophages **(black arrows)**.

The fusion of macrophages can occur as a result of chronic inflammation. PD-L1 staining multi-nucleate macrophages (giant cells) should be counted as one cell and included in the numerator.

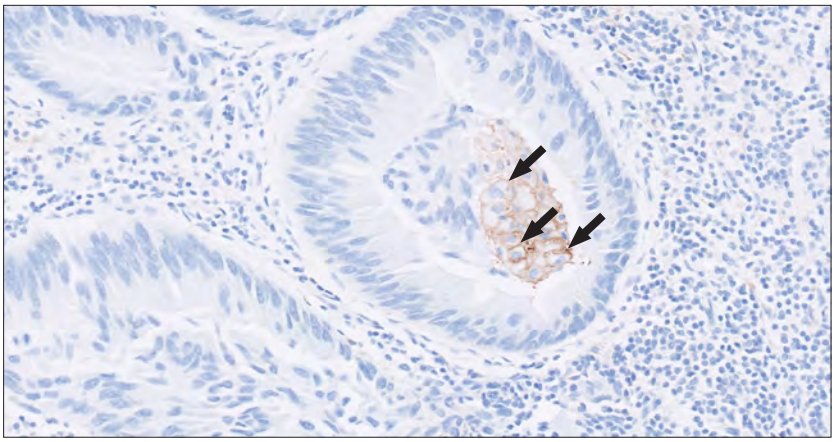


Figure 24. 20x magnification.

Key highlights

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

20x Field of View Rule

PD-L1 staining MICs must be directly associated with the response against the tumor to be included in the CPS numerator. MICs are considered tumor-associated if they are present within the tumor nests and/or adjacent supporting stroma within a 20x magnification field of view. In cases where it is difficult to tell if MICs are tumor-associated, the following is suggested as a guideline: Move the slide so that the tumor is in the approximate center of a 20x field. Immune cells surrounding the tumor in this field should be included in the scoring. Immune cells outside of this field should be excluded from scoring as long as they do not surround neighboring tumor cells. In general, include PD-L1 staining MICs that are within 0.5 mm of the tumor cells. This rule may be applied to tumors within lymph nodes that contain PD-L1 staining MICs. See Figures 25 and 27 for an example of determining which MICs are included in the CPS numerator.

GEJ SQCC specimen stained with PD-L1 primary antibody. At 5x magnification, several areas of staining MICs are visible. To demonstrate which immune cells to include in the numerator, zoom in to 20x magnification on the boxed fields.

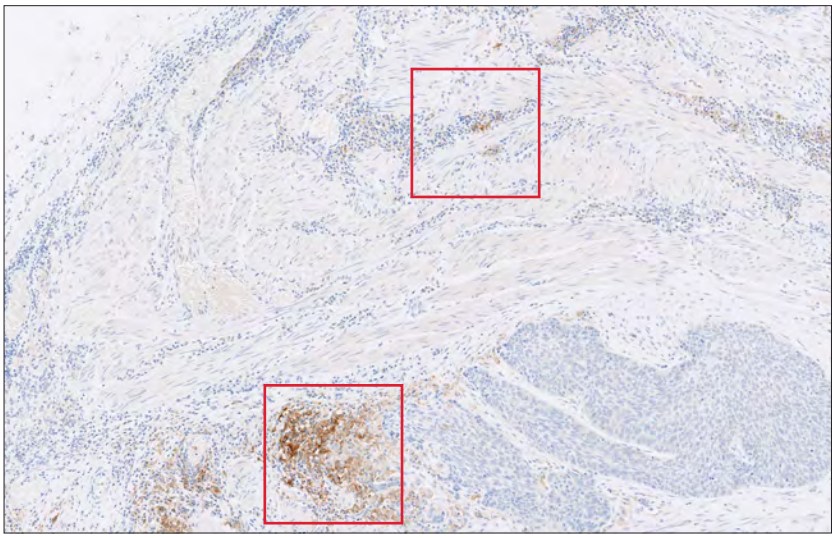


Figure 25. 5x magnification.

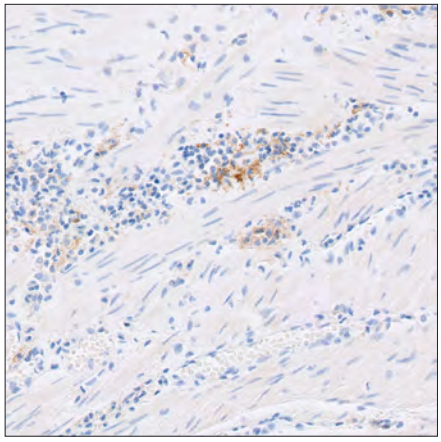


Figure 26. 20x magnification.

Tumor cells are absent from this 20x field containing PD-L1 staining mononuclear inflammatory cells, thus these cells should be excluded from the numerator.

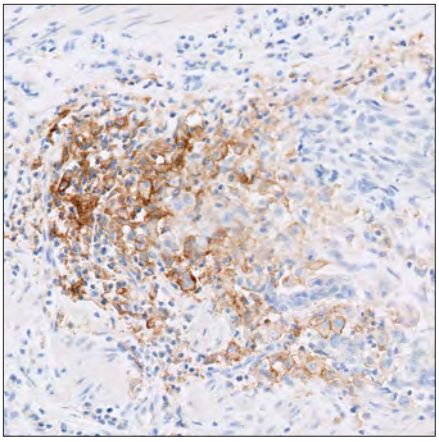


Figure 27. 20x magnification.

When positioning the tumor cells in the approximate center of a 20x field, PD-L1 staining mononuclear inflammatory cells that are present within the same field should be included in the numerator.

Cells excluded from CPS

Only tumor cells exhibiting PD-L1 membrane staining and MICs exhibiting PD-L1 membrane and/or cytoplasmic staining should be included in the CPS numerator. Below are common examples of other cells that can exhibit PD-L1 expression but should be excluded from the CPS calculation (CPS numerator and/or denominator). For complete inclusion/exclusion criteria refer to Tables 2 and 3 on page numbers 24 and 25 respectively.

Tumor cells with cytoplasmic stain only

EC SQCC specimen stained with PD-L1 primary antibody exhibiting 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.

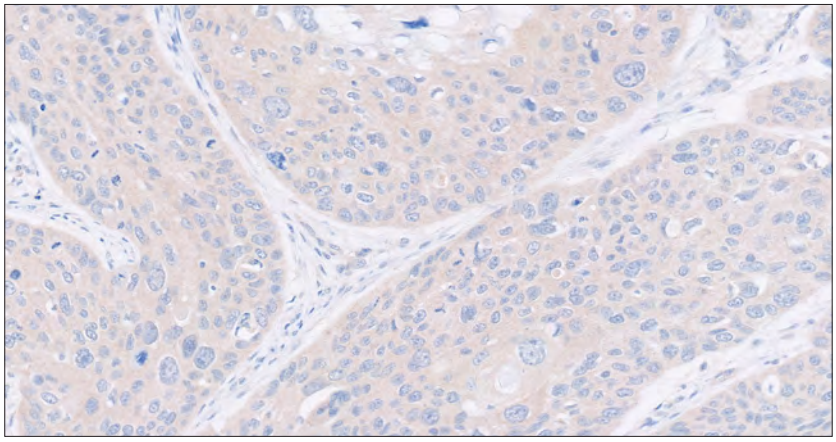


Figure 28. 20x magnification.

Tumor cells with indeterminant cytoplasmic stain

GC specimen stained with PD-L1 primary antibody exhibiting strong cytoplasmic and membrane staining. Some membrane can be distinguished from the cytoplasmic background (red arrows) (20x magnification).

Membrane staining occluded by strong cytoplasmic staining should be excluded from CPS numerator and denominator. If the majority of membrane staining is occluded by cytoplasmic staining, then the specimen should be deemed non-evaluable (NE).

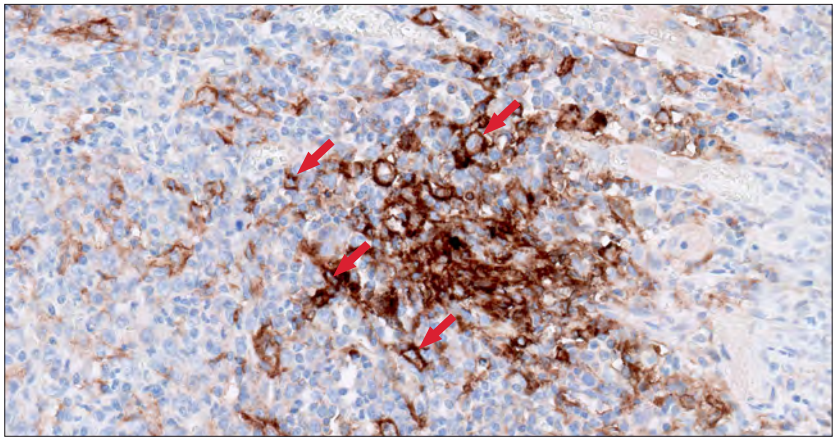


Figure 29. 20x magnification.

Key highlights

- Tumor cells exhibiting only cytoplasmic staining should not be included in the CPS numerator
- Membrane staining occluded by strong cytoplasmic staining should be excluded from the CPS numerator and denominator

Carcinoma in situ

H&E section of an EC SQCC specimen demonstrating carcinoma in situ (CIS). The boxed region is shown below at higher magnification.

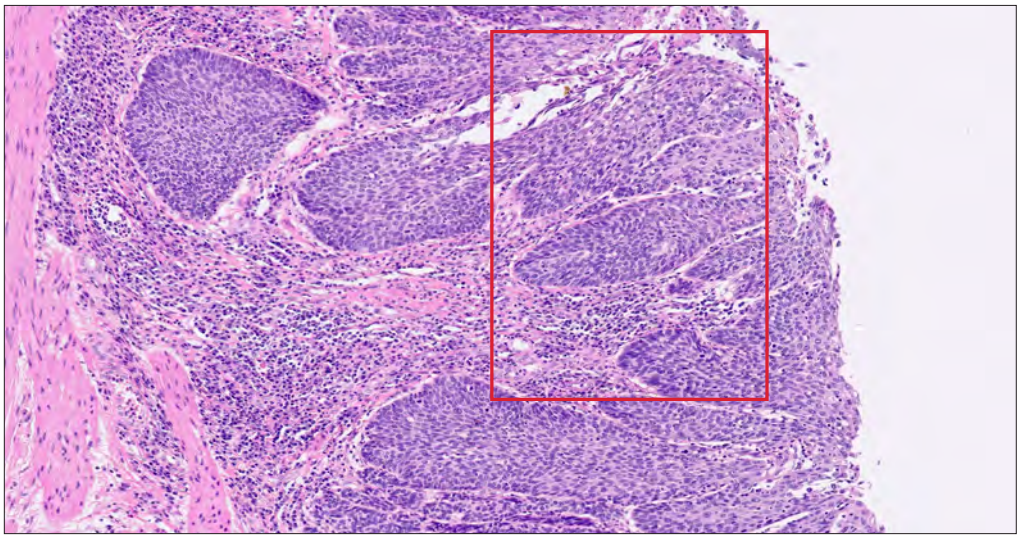


Figure 30. 5x magnification.

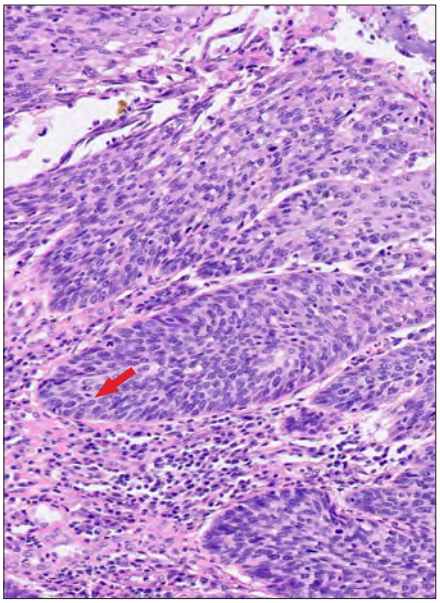


Figure 31. 10x magnification.

H&E section demonstrating tumor cells involved in a CIS component (red arrow).

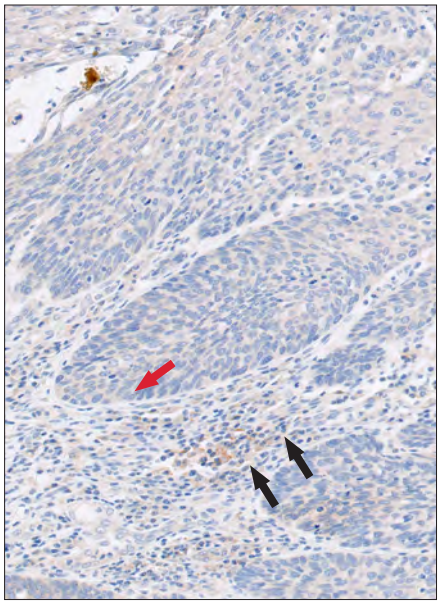


Figure 32. 10x magnification.

Any tumor cells that are part of the CIS component should be excluded from the numerator and denominator (red arrows). Any MICs (black arrows) associated with the CIS component should be excluded from the numerator.

Key highlights

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

MICs associated with normal gastric glands

GC specimen stained with PD-L1 primary antibody shows staining of MICs associated with normal gastric glands.

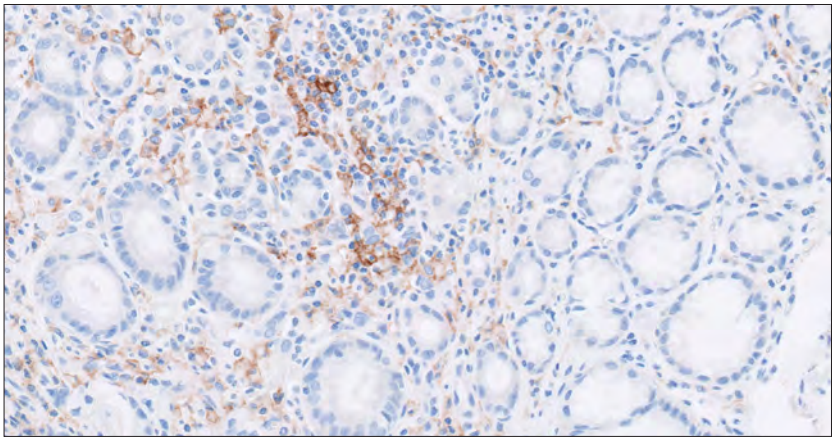


Figure 33. 10x magnification.

MICs contributing to gastritis

GEJ specimen stained with PD-L1 primary antibody shows staining of MICs contributing to gastritis. Notice the increase in plasma cells which is a feature of chronic gastritis.

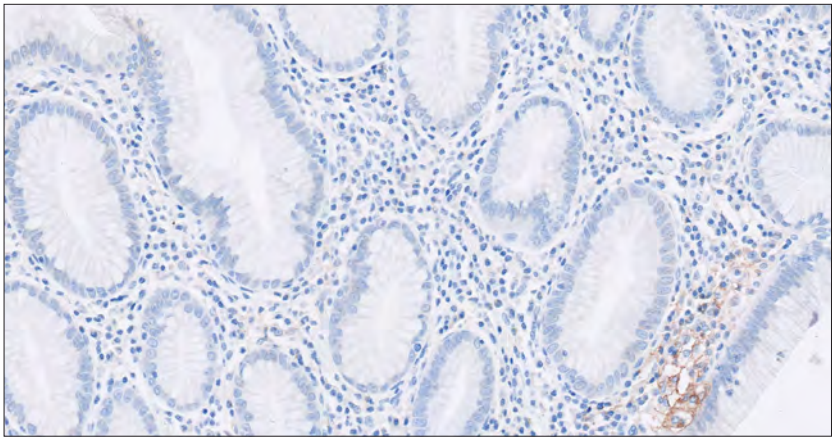


Figure 34. 20x magnification.

MICs associated with non-invasive dysplasia

GEJ specimen stained with PD-L1 primary antibody, shows staining of MICs associated with glandular dysplasia.

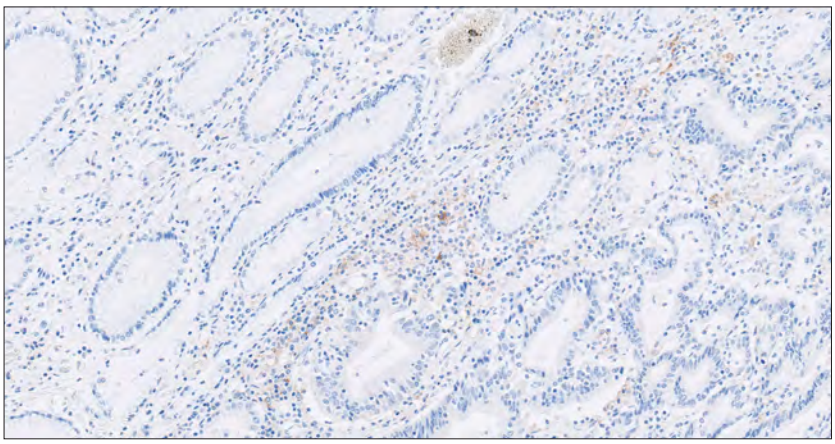


Figure 35. 10x magnification.

Key highlights

PD-L1 staining MICs unassociated with the direct tumor response should be excluded from the numerator

Staining of benign gastric glands

GC specimen stained with PD-L1 primary antibody shows staining of benign gastric glands. Staining of benign gastric glands should be excluded from CPS.

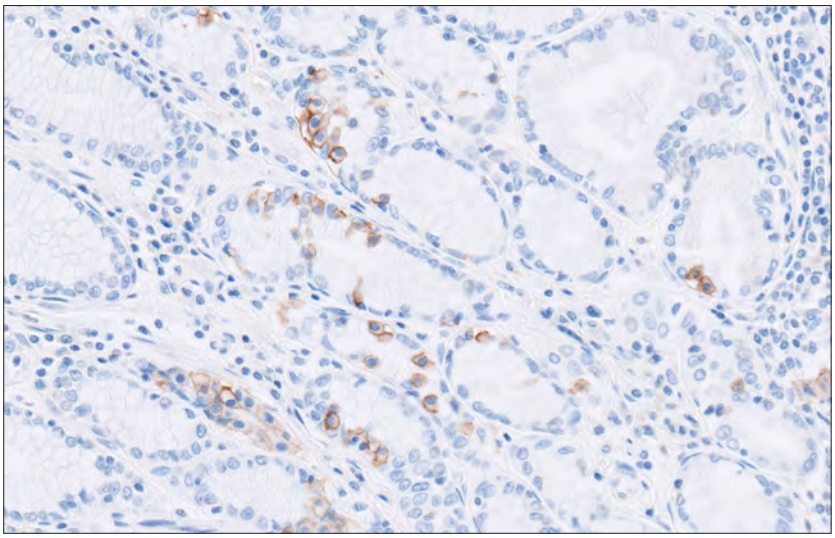


Figure 36. 20x magnification.

Staining of benign glandular cells

GC specimen stained with PD-L1 primary antibody shows staining of invasive tumor cells (black arrows), benign glandular cells (red arrow) and endocrine cells (blue arrow). Staining of benign glandular cells and endocrine cells should be excluded from CPS.

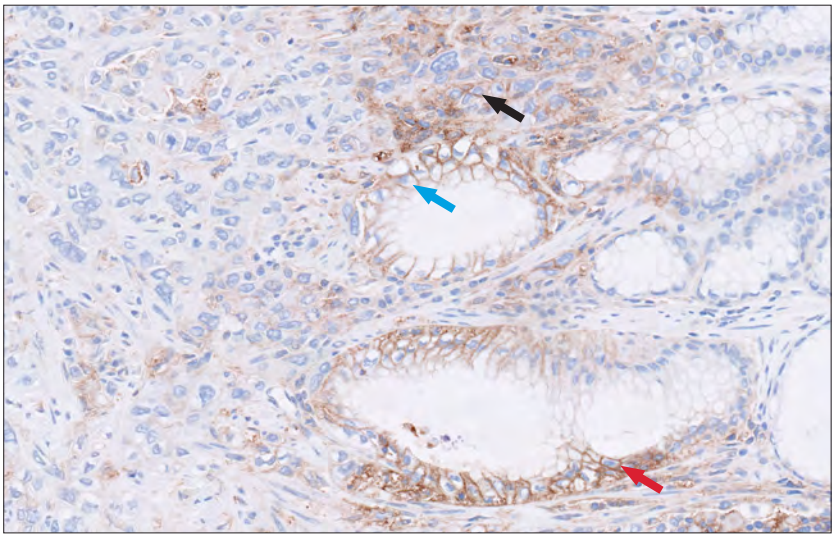


Figure 37. 20x magnification.

Key highlights

PD-L1 staining of benign cells should be excluded from the numerator

Staining of fibroblasts

GC specimen stained with PD-L1 primary antibody exhibiting staining of fibroblasts **(black arrows)**, which should be excluded from the CPS.

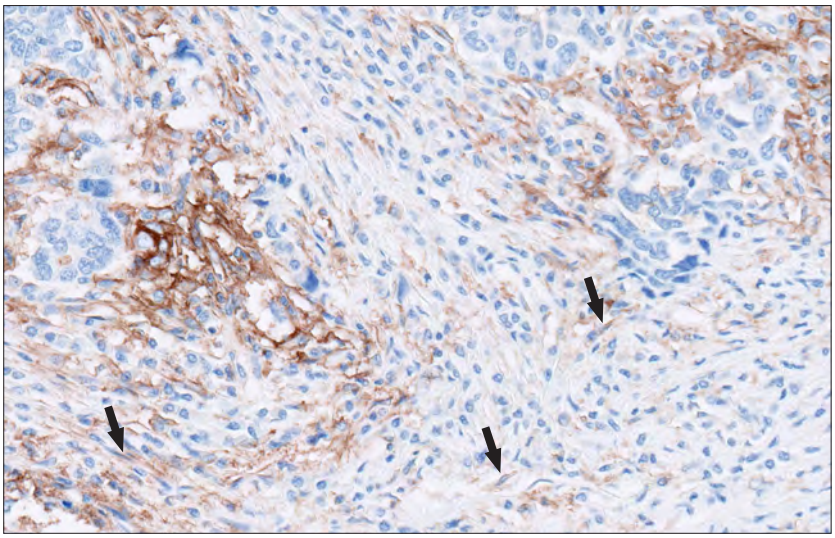


Figure 38. 20x magnification.

Staining of ganglion cells

GEJ specimen stained with PD-L1 primary antibody exhibiting staining of ganglion cells **(black arrows)** which should be excluded from the CPS.

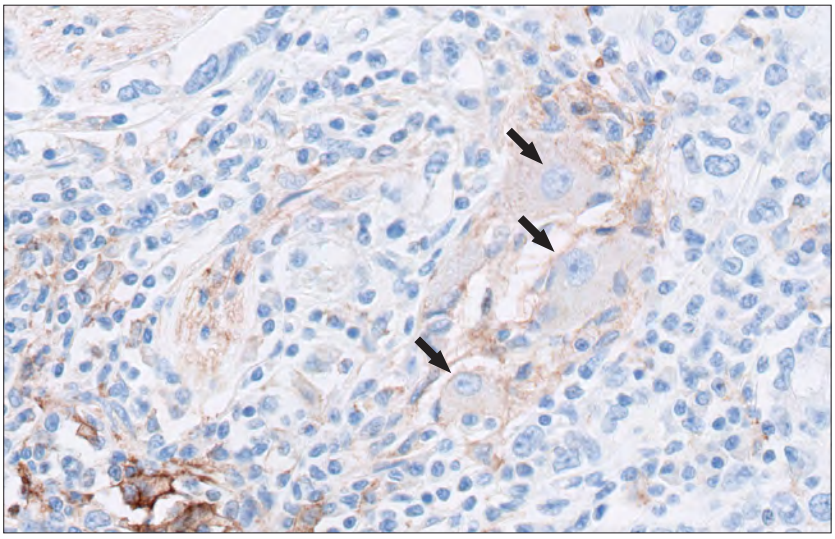


Figure 39. 20x magnification.

Staining normal epithelium

GEJ specimen stained with PD-L1 primary antibody exhibiting staining of benign epithelial cells (red arrows) and associated MICs (**black arrows**), both of which should be excluded from the score.

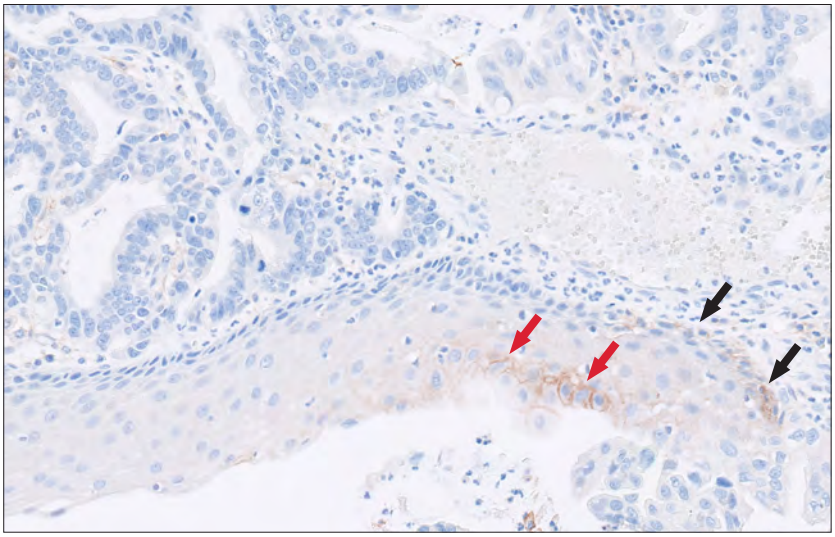


Figure 40. 20x magnification.

Staining ulcerated epithelium

GEJ SQCC specimen stained with PD-L1 primary antibody exhibiting staining of benign ulcerated epithelial cells (red arrows) and associated MICs (**black arrows**), both of which should be excluded from the score.

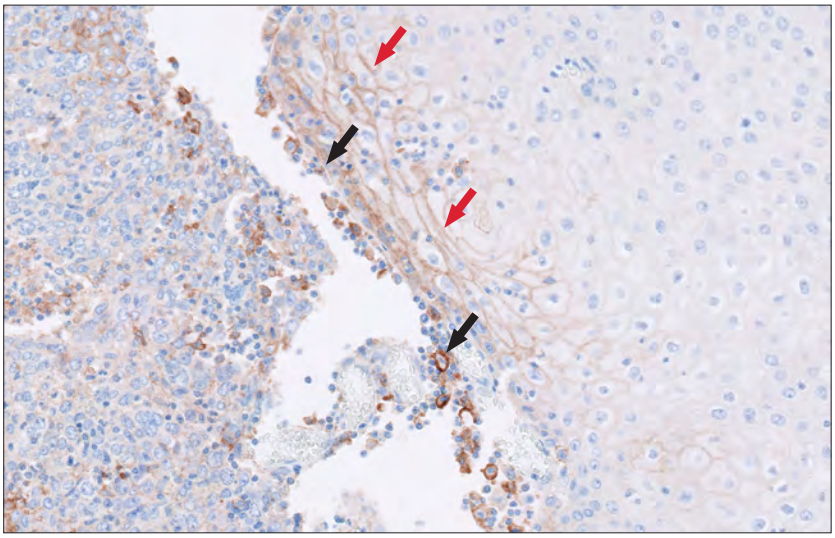


Figure 41. 20x magnification.

Key highlights

- PD-L1 staining of normal cells should be excluded from the numerator and denominator
- PD-L1 staining of MICs unassociated with the direct tumor response should be excluded from the numerator

Immune cells excluded from CPS

Plasma cells

GC specimen stained with PD-L1 primary antibody exhibiting staining of plasma cells **(black arrows),** which should be excluded from the score.

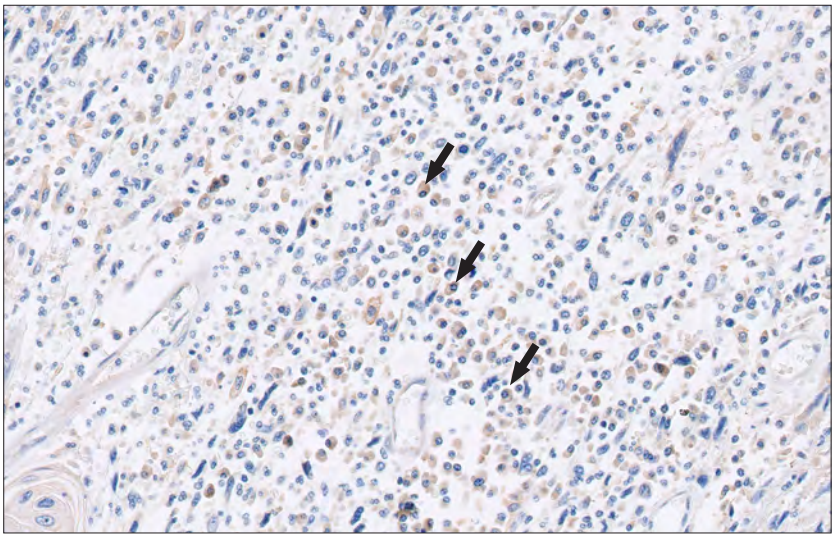


Figure 42. 20x magnification.

Neutrophils

EC SQCC specimen stained with PD-L1 primary antibody exhibiting staining of neutrophils **(black arrows),** which should be excluded from the score.

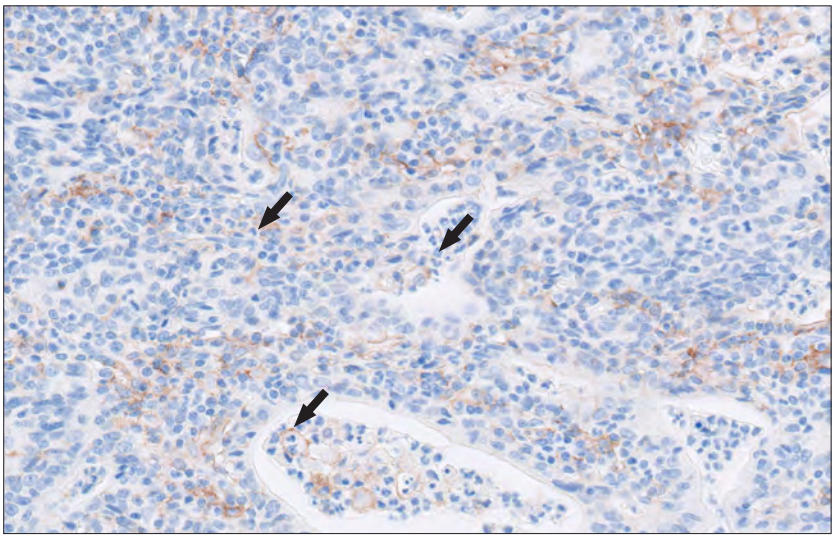


Figure 43. 20x magnification.

Key highlights

- Only lymphocytes and macrophages with cytoplasmic and/or membrane staining are included in the numerator
- Plasma cells, neutrophils, and eosinophils are excluded from the CPS numerator

PD-L1 IHC 28-8 pharmDx Gastric, GEJ, and Esophageal Adenocarcinoma

CPS 0 case examples

GC specimen stained with PD-L1 antibody exhibiting CPS 0.

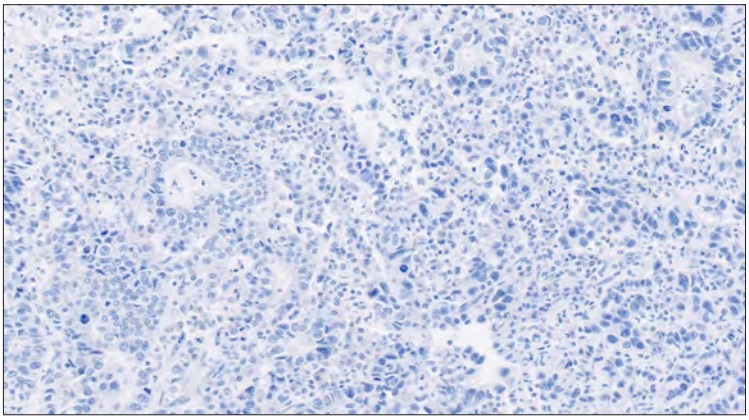


Figure 44. 20x magnification

GEJ specimen stained with PD-L1 antibody exhibiting CPS 0.

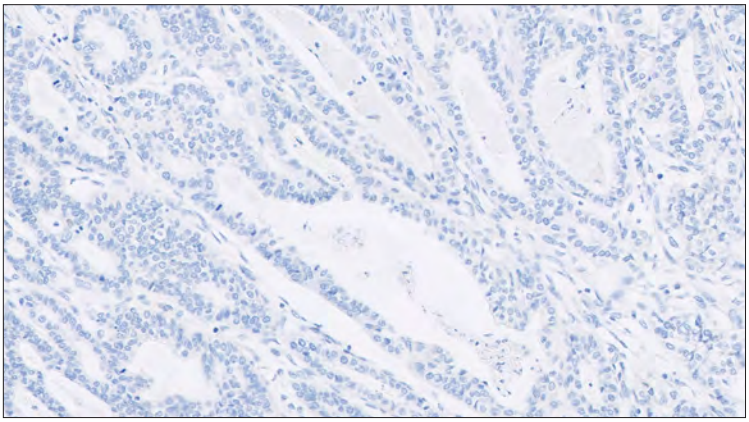


Figure 45. 20x magnification

EC SQCC specimen stained with PD-L1 antibody exhibiting CPS 0.

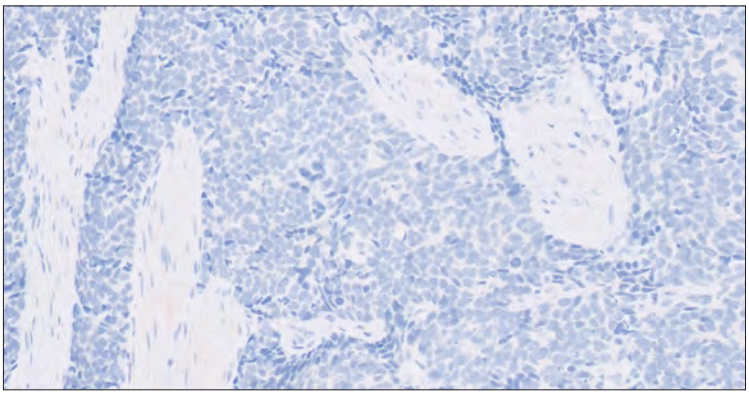


Figure 46. 20x magnification

Challenging cases: CPS < 1

GC specimen stained with PD-L1 antibody exhibiting CPS < 1.

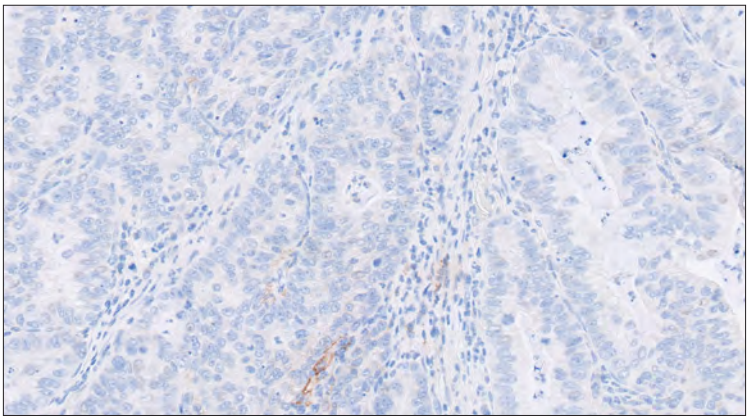


Figure 47. 20x magnification

GEJ specimen stained with PD-L1 antibody exhibiting CPS < 1.

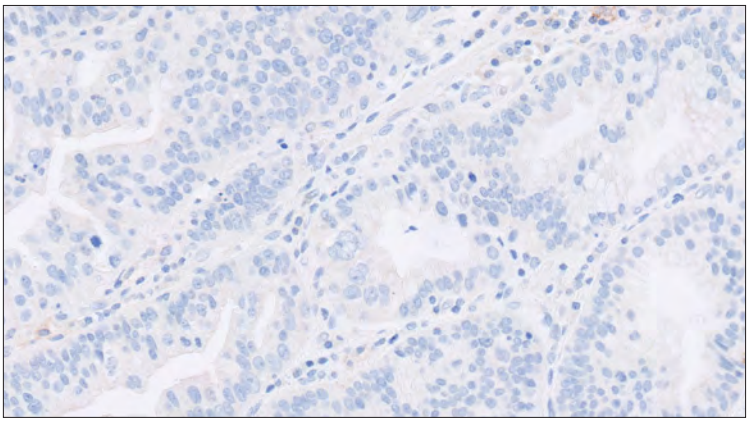


Figure 48. 20x magnification

EAC specimen stained with PD-L1 antibody exhibiting CPS < 1.

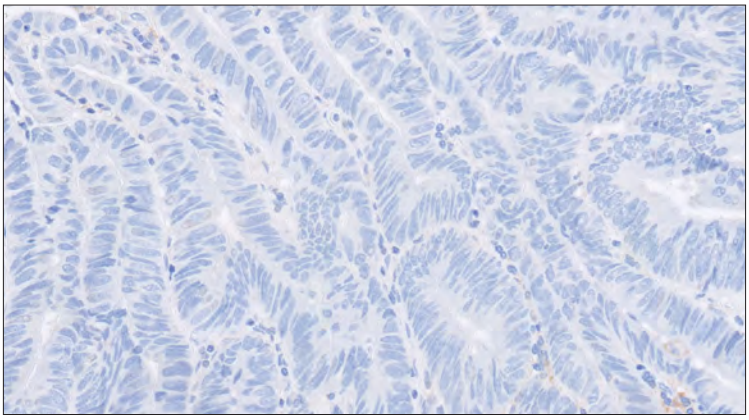


Figure 49. 20x magnification

Challenging cases: CPS 1–4

GC specimen stained with PD-L1 antibody exhibiting CPS 1.

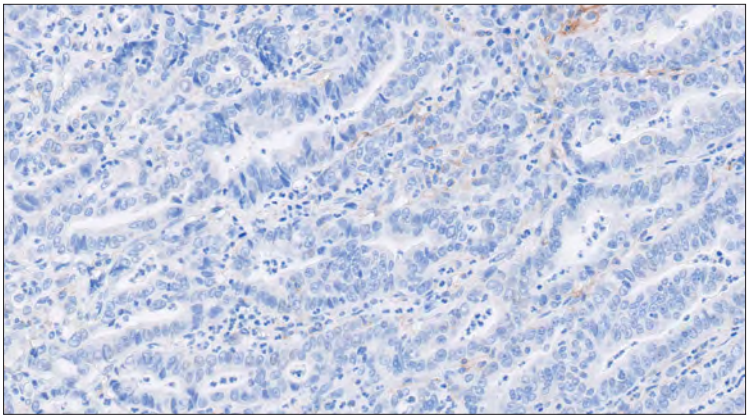


Figure 50. 20x magnification

GEJ SQCC specimen stained with PD-L1 antibody exhibiting CPS 3.

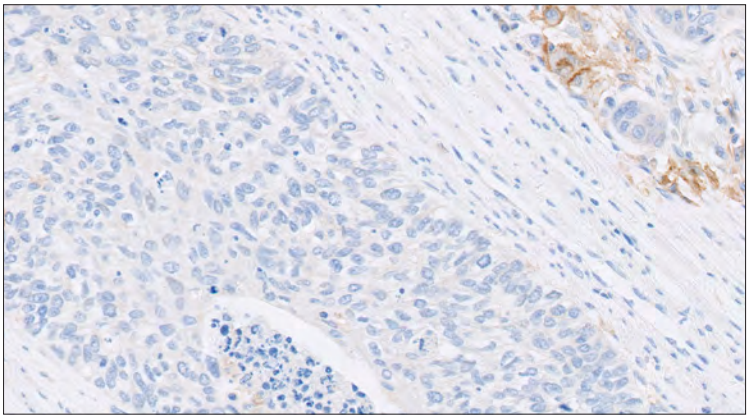


Figure 51. 20x magnification

EAC specimen stained with PD-L1 antibody exhibiting CPS 2.

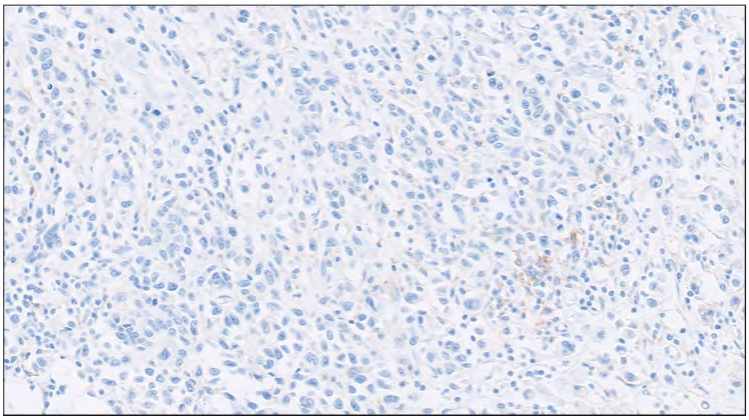


Figure 52. 20x magnification

Challenging cases: CPS 1–4

GC specimen stained with PD-L1 antibody exhibiting CPS 1.

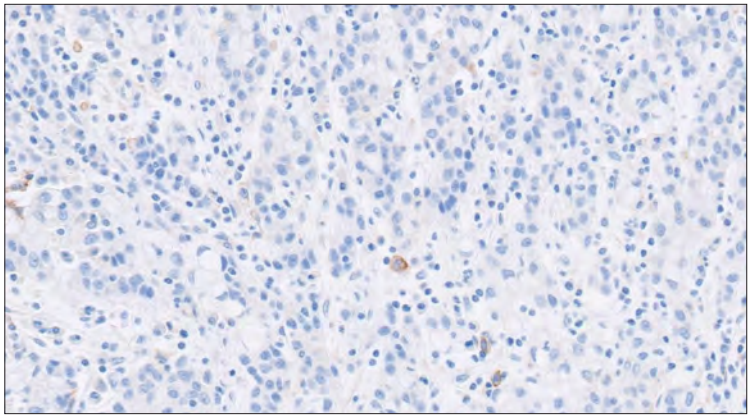


Figure 53. 20x magnification

GEJ specimen stained with PD-L1 antibody exhibiting CPS 2.

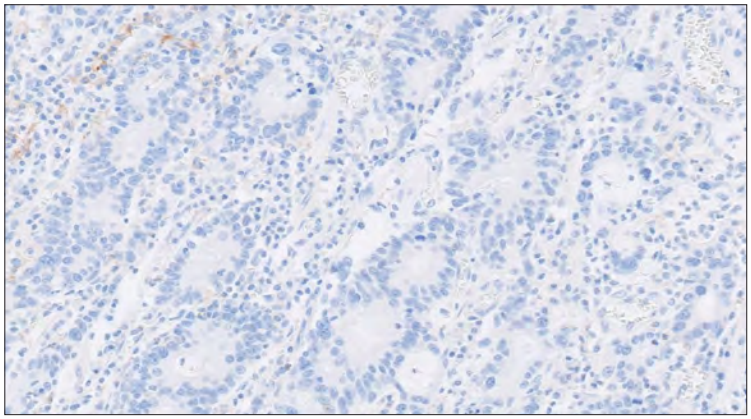


Figure 54. 20x magnification

Challenging cases: CPS 5–10.

GC specimen stained with PD-L1 antibody exhibiting CPS 7.

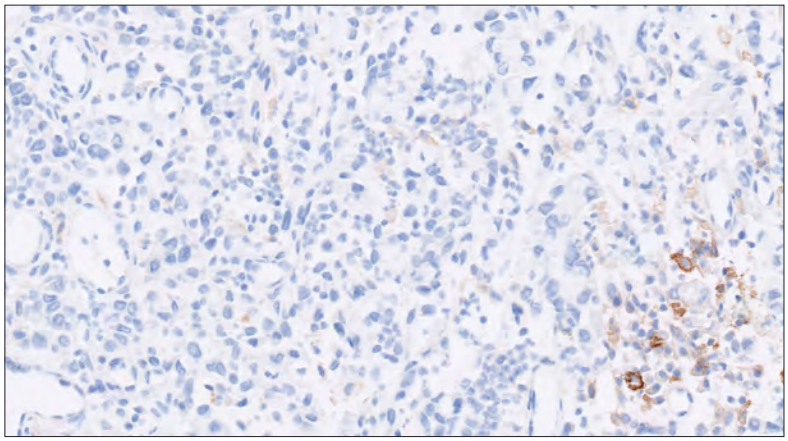


Figure 55. 20x magnification

GEJ specimen stained with PD-L1 antibody exhibiting CPS 6.

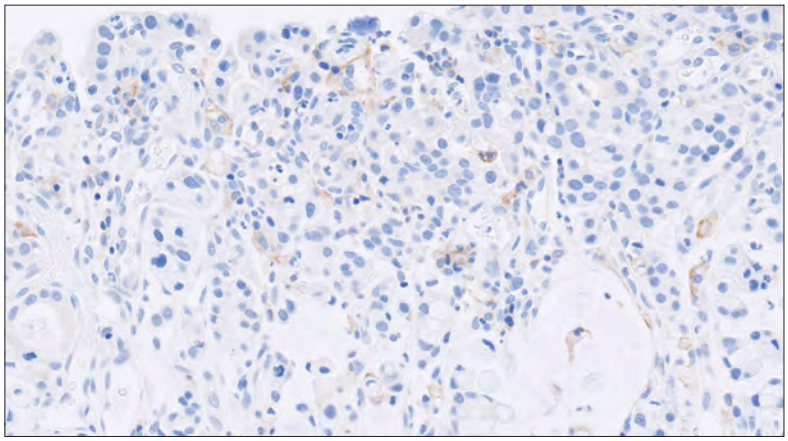


Figure 56. 20x magnification

EAC specimen stained with PD-L1 antibody exhibiting CPS 5.

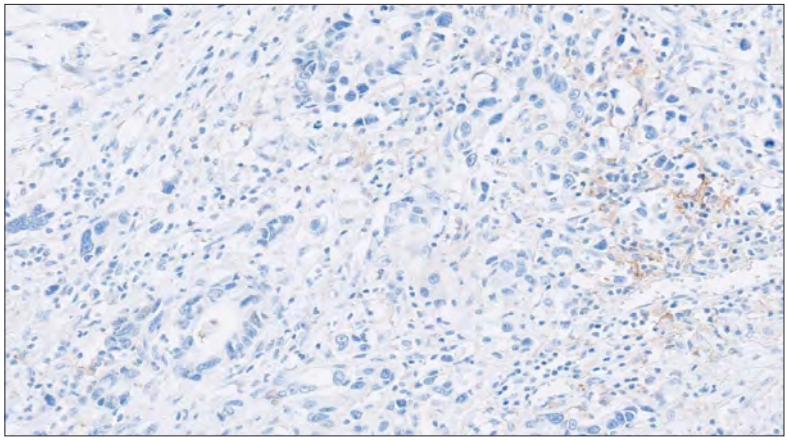


Figure 57. 20x magnification

Challenging cases: CPS 5-10

GC specimen stained with PD-L1 antibody exhibiting CPS 8.

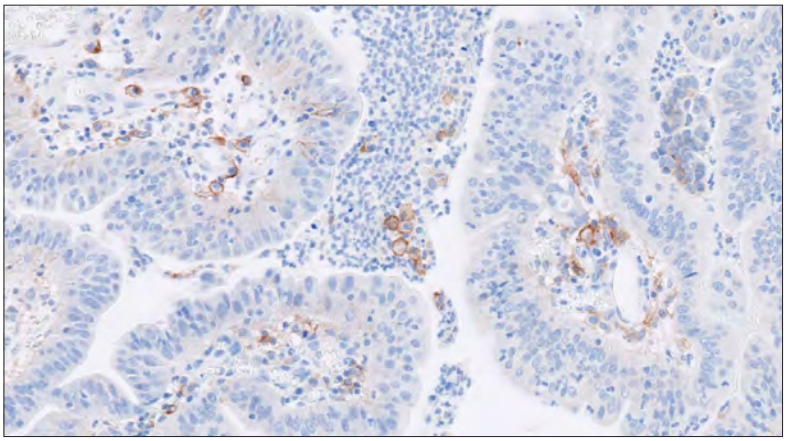


Figure 58. 20x magnification

GEJ specimen stained with PD-L1 antibody exhibiting CPS 10.

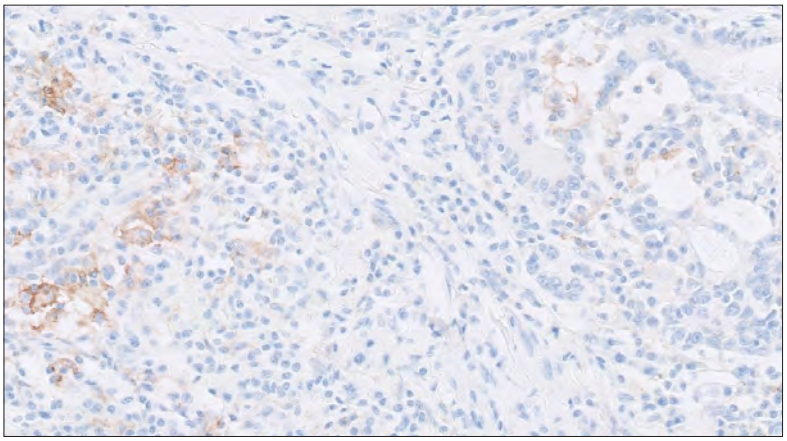


Figure 59. 20x magnification

Case examples: CPS >10 (High positive)

GC specimen stained with PD-L1 antibody exhibiting CPS >10. Any CPS score between CPS 50–60 may be assigned to this specimen.

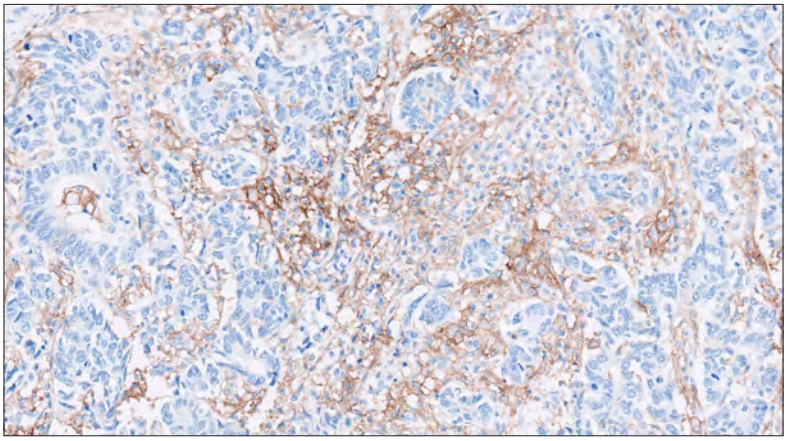


Figure 60. 20x magnification

GEJ specimen stained with PD-L1 antibody exhibiting CPS >10. Any CPS score between CPS 20–30 may be assigned to this specimen.

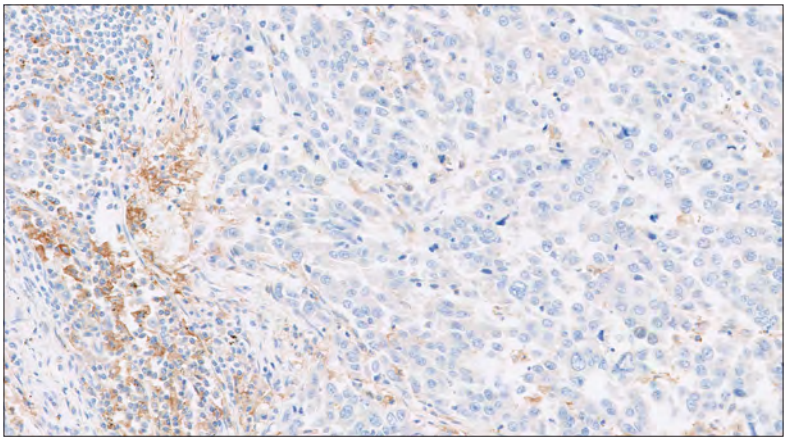


Figure 61. 20x magnification

EAC specimen stained with PD-L1 antibody exhibiting CPS 30.

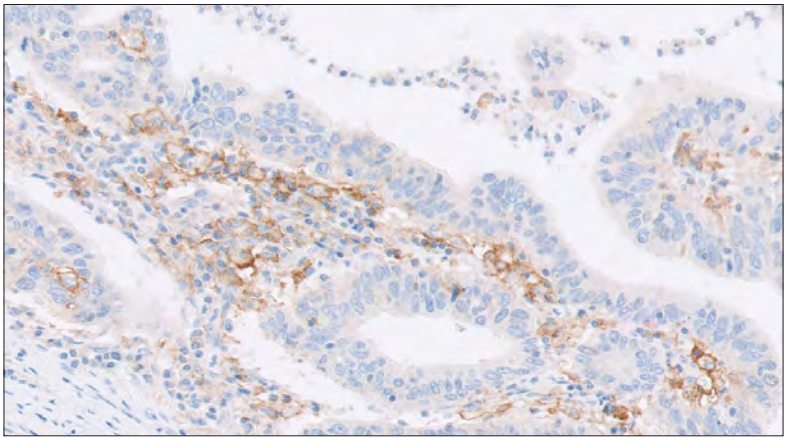


Figure 62. 20x magnification

Artifacts

Non-specific staining

Non-specific staining is defined as any off target staining of the specimen and is often diffuse in pattern. It is caused by several factors. These factors include, but are not limited to, pre-analytic fixation and processing of the specimen, incomplete removal of paraffin from sections, and incomplete rinsing of slides.

The use of fixatives other than 10% NBF may be a source of non-specific staining.

Possible causes of non-specific staining

- Improper drying of slides; ensure slides remain wet with buffer while loading onto Autostainer Link 48 and prior to initiating run
- Improper deparaffinization procedure
- Incomplete rinsing of reagents from slides
- Improper mixing of wash buffer

The non-specific staining present on the negative control tissue specimen is useful in determining the level of non-specific staining in the same patient tissue specimen stained with PD-L1. All specimens must have ≤ 1+ non-specific staining.

GEJ specimen stained with PD-L1 primary antibody exhibiting non-specific staining; cells with non-specific staining should be excluded from the score.

Non-specific staining can be seen here in the cytoplasm of tumor cells, and stroma of the tissue (red arrows).

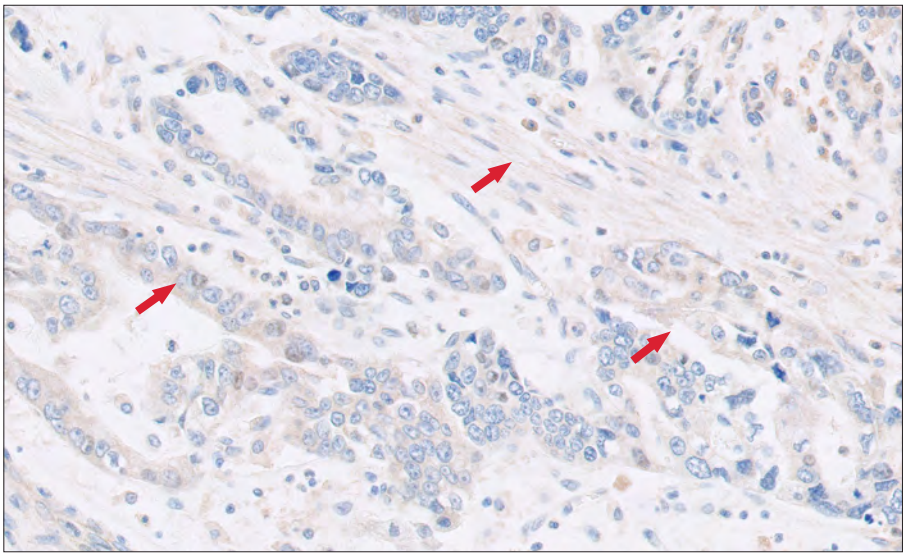


Figure 63. 20x magnification.

DAB staining

EC SQCC specimen stained with PD-L1 primary antibody exhibiting non-specific DAB staining; non-specific DAB staining should be excluded from the score.

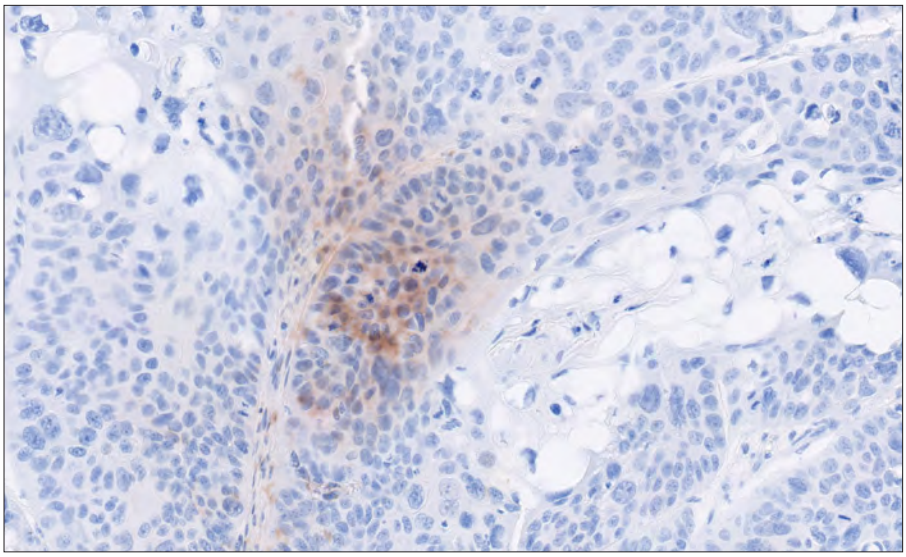


Figure 64. 20x magnification.

Poor fixation

EAC specimen stained with PD-L1 primary antibody exhibiting poor tissue fixation. Larger resection specimens commonly seen in GC, GEJ, and EAC can suffer from poor fixation issues. These issues can cause poor tissue integrity, impaired morphology, and loss of antigen leading to an incorrect diagnosis of PD-L1 expression.

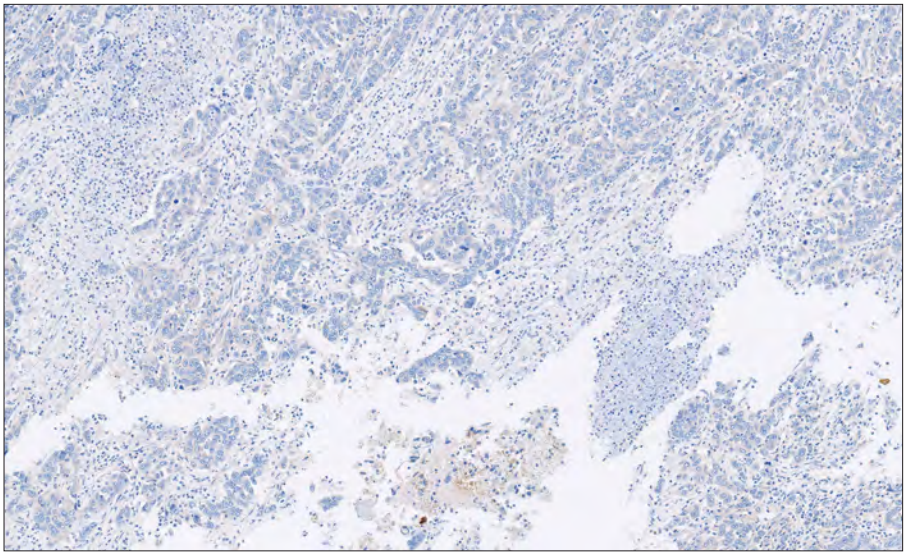


Figure 65. 10x magnification.

Edge effect

GEJ specimen stained with PD-L1 primary antibody exhibiting edge effect; edge staining should be excluded from the score. Edge effect can be more convincingly called if the central tumor mass is completely negative or shows significantly lower staining.

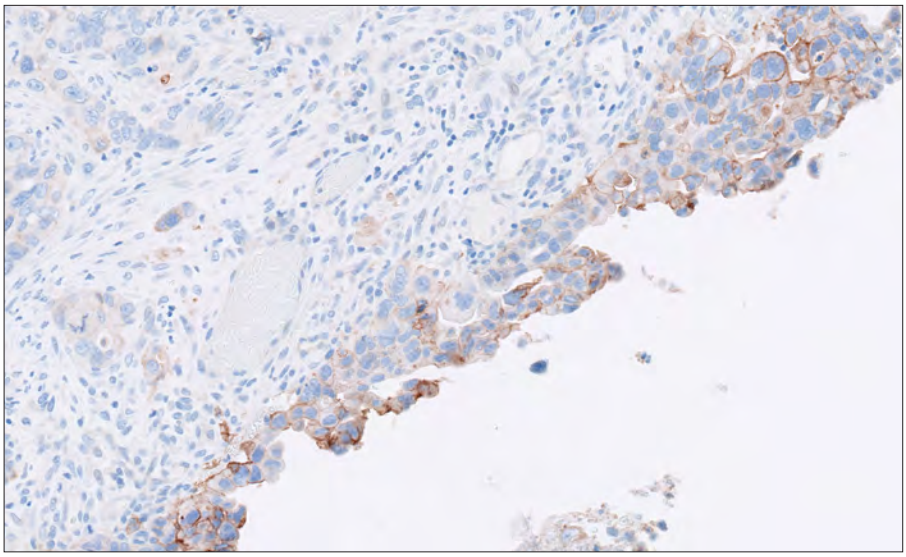


Figure 66. 20x magnification.

Crush artifact

EAC specimen stained with PD-L1 primary antibody exhibiting crush artifact; crush artifact should be excluded from the score.

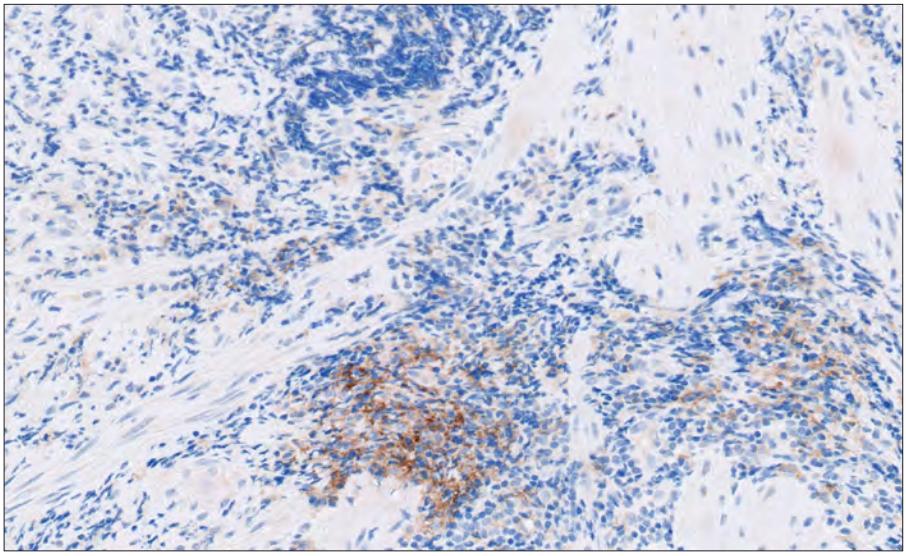


Figure 67. 20x magnification.

Necrosis

Necrotic tissue may show non-specific staining and should not be included in CPS evaluation. Care should be taken to only include viable tumor cells for scoring and not necrotic regions.

GC specimen stained with PD-L1 primary antibody exhibiting staining of necrosis; necrosis staining should be excluded from the score.

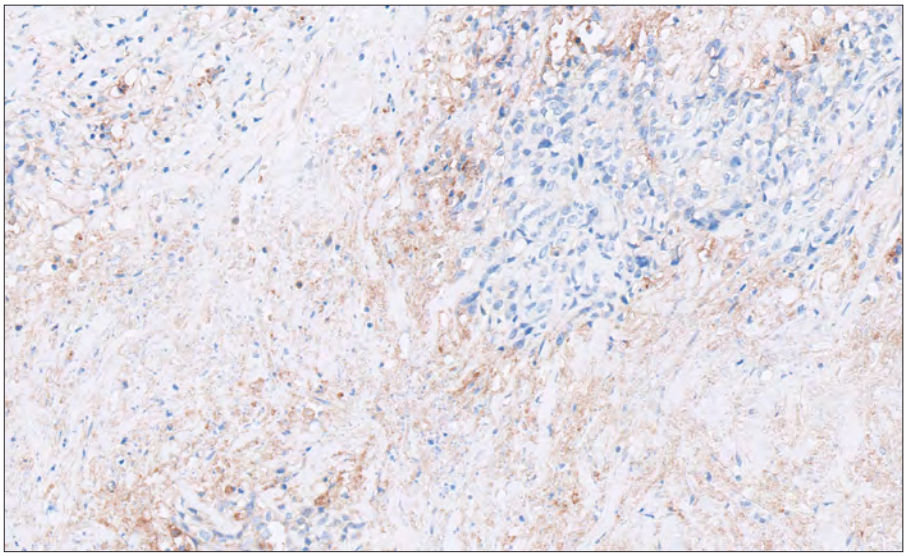


Figure 68. 20x magnification.

Additional – Subhistologies

GC specimen stained with PD-L1 showing well-differentiated adenocarcinoma.

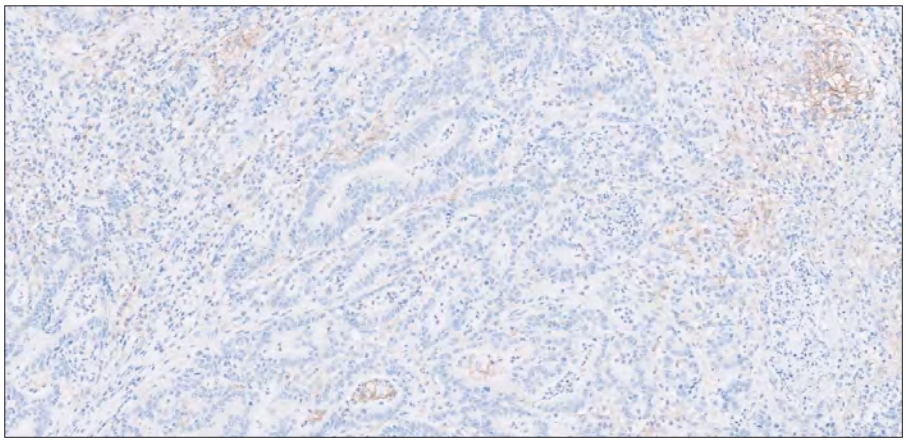


Figure 69a. 10x magnification.

GEJ specimen stained with PD-L1 showing moderately to poorly differentiated adenocarcinoma and squamous cell carcinoma component.

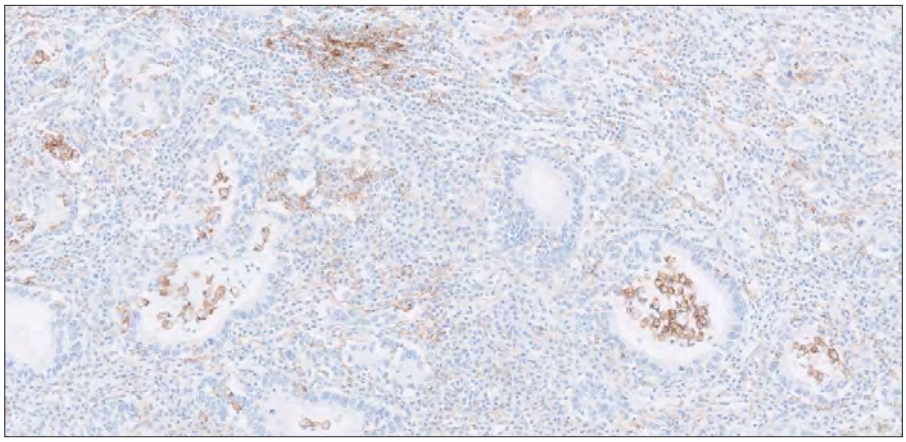


Figure 69b. 10x magnification.

EAC specimen stained with PD-L1 showing squamous cell carcinoma component.

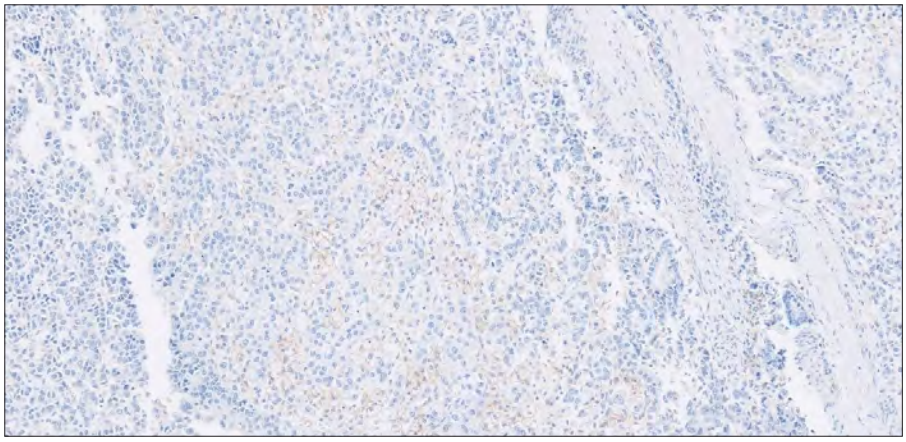


Figure 69c. 10x magnification.

Disclaimer: Examples of squamous cell carcinoma (SQCC) specimens are included for gastroesophageal junction carcinoma and esophageal carcinoma as representations of SQCC components that can be seen within adenocarcinoma specimens. SQCC specimens are not part of the test labeling.

GC specimen stained with PD-L1 showing mucinous carcinoma characterized by substantial extracellular mucin pools.

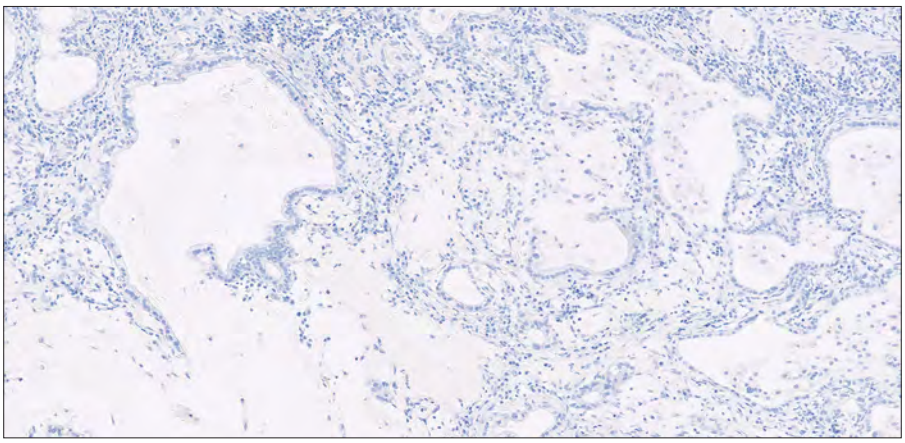


Figure 70a. 10x magnification.

GEJ specimen stained with PD-L1 antibody with signet ring cell morphology.

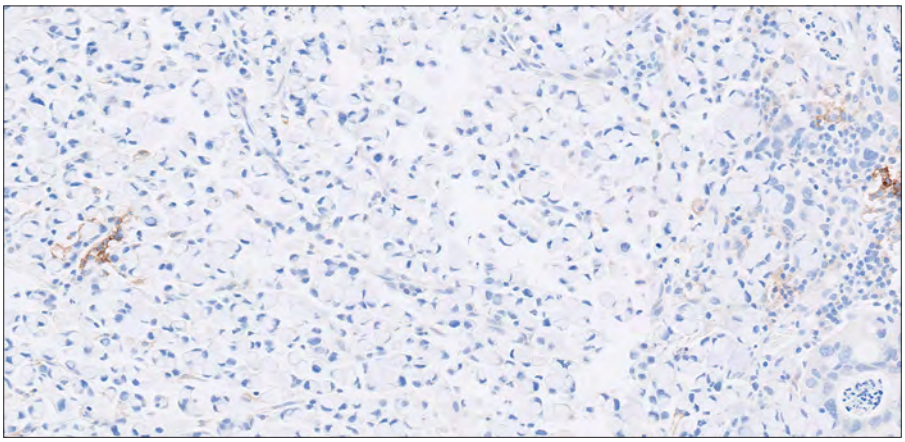


Figure 70b. 20x magnification.

Troubleshooting Guide for PD-L1 IHC 28-8 pharmDx

Problem	Probable Cause	Suggested Action
1. No staining of control or specimen slides	1a. Programming error	1a. Verify that the SK005 PD-L1 IHC 28-8 pharmDx program was selected for programming of slides
	1b. Lack of reaction with DAB+ Substrate - Chromogen Solution (DAB)	1b. Verify that DAB+ Substrate-Chromogen Solution was prepared properly
	1c. Sodium azide in wash buffer	1c. Use only Dako Wash Buffer (Code K8007)
	1d. Degradation of Control Slide	1d. Check kit expiration date and kit storage conditions on outside of package
2. Weak staining of specimen slides	2a. Inappropriate fixation method used	2a. Ensure that only neutral buffered formalin fixative and approved fixation methods are used
	2b. Insufficient reagent volume applied	2b. Check size of tissue section and reagent volume applied
	2c. Inappropriate wash buffer used	2c. Use only Dako Wash Buffer (Code K8007)
3. Weak staining of specimen slides or the positive cell line on the Dako-supplied Control Slide	3a. Inadequate Target Retrieval	3a. Verify that the 3-in-1 pre-treatment procedure was correctly performed
	3b. Inappropriate wash buffer used	3b. Use only Dako Wash Buffer (Code K8007)
4. Excessive non-specific staining of slides	4a. Paraffin incompletely removed	4a. Verify that the 3-in-1 pre-treatment procedure was correctly performed
	4b. Slides dried while loading onto the Autostainer Link 48	4b. Ensure slides remain wet with buffer while loading and prior to initiating run
	4c. Non-specific binding of reagents to tissue section	4c. Check for proper fixation of the specimen and/or the presence of necrosis
	4d. Inappropriate fixation method used	4d. Ensure that only neutral buffered formalin fixative and approved fixation methods are used
	4e. Inadequate mixing of wash buffer	4e. Ensure wash buffer is properly mixed
5. Tissue detached from slides	5a. Use of incorrect microscope slides	5a. Use Dako FLEX IHC Microscope Slides (Code K8020), or Superfrost Plus slides
	5b. Inadequate preparation of specimens	5b. Cut sections should be placed in a 58 ± 2 °C oven for 1 hour prior to staining
6. Excessively strong specific staining	6a. Inappropriate fixation method used	6a. Ensure that only approved fixatives and fixation methods are used
	6b. Inappropriate wash buffer used	6b. Use only Dako Wash Buffer (Code K8007)

Problem	Probable Cause	Suggested Action
7. Target Retrieval Solution is cloudy in appearance when heated	7. When heated the Target Retrieval Solution turns cloudy in appearance	7. This is normal and does not influence staining
8. 1x Target Retrieval Solution does not meet pH specifications	8a. pH meter is not calibrated correctly	8a. Ensure pH meter is calibrated per manufacturer's recommendations. After re-calibration, re-test the pH of 1x Target Retrieval Solution. Do not modify the pH of 1x Target Retrieval Solution. If the pH is outside the acceptable range (6.1 ± 0.2), discard 1x Target Retrieval Solution. Prepare new 1x Target Retrieval Solution. Check the pH of the new 1x Target Retrieval Solution
	8b. Inferior quality water is used to dilute the Target Retrieval Solution concentrate	8b. Ensure that distilled or deionized water is used to prepare 1x Target Retrieval Solution
	8c. Incorrect Target Retrieval Solution is used	8c. Ensure that the correct EnVision Flex Target Retrieval Solution specified in "Materials Provided" and "Reagent Preparation" sections of the IFU is used

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