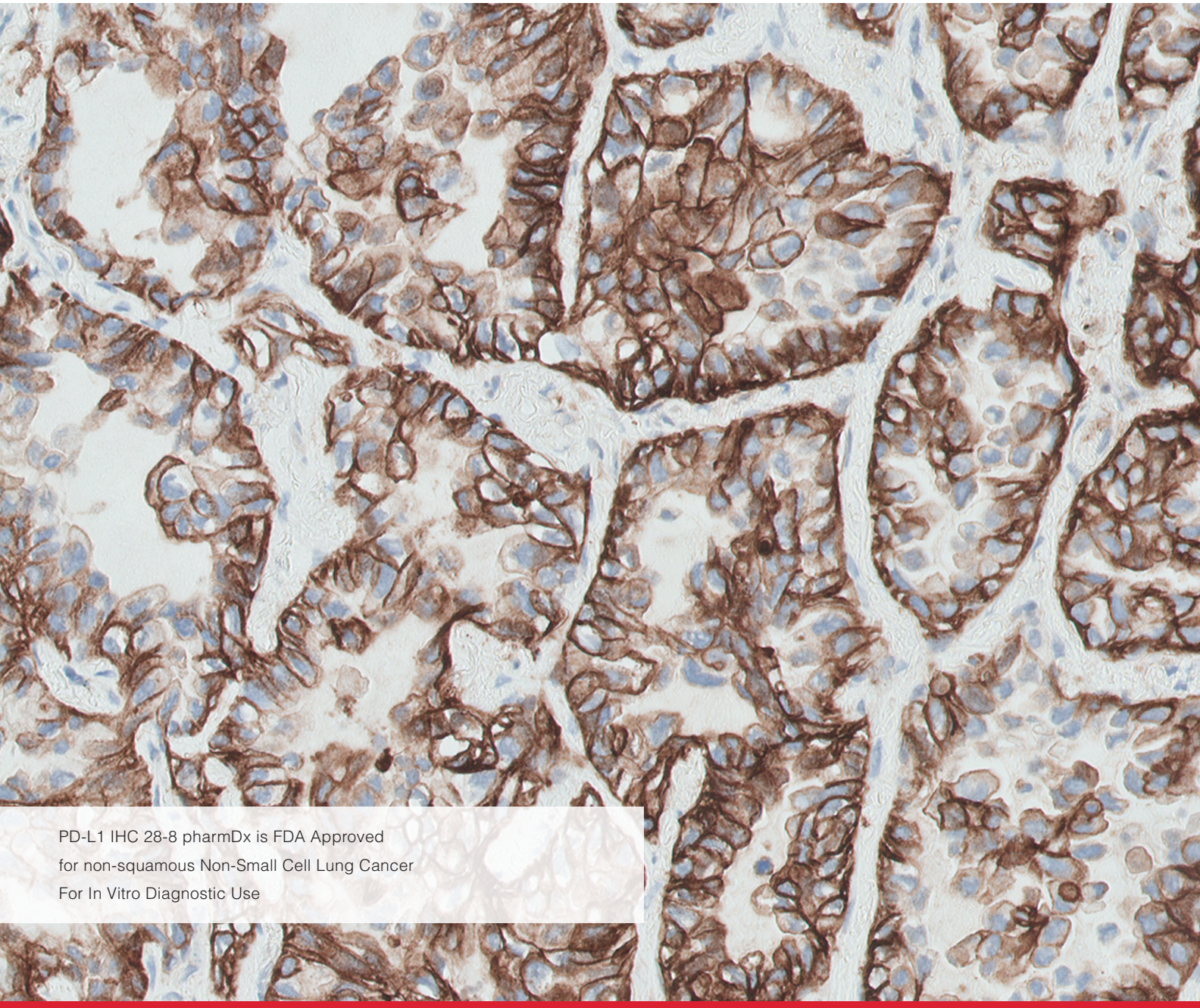
EDUCATION

PD-L1 IHC 28-8 pharmDx | Interpretation Manual Non-Squamous Non-Small Cell Lung Cancer

Dako

An **Agilent Technologies** Company



PD-L1 IHC 28-8 pharmDx is FDA Approved
for non-squamous Non-Small Cell Lung Cancer
For In Vitro Diagnostic Use

Agilent Technologies

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Introduction

PD-L1 IHC 28-8 pharmDx Intended 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 (NSCLC) tissue using EnVision FLEX visualization system on Autostainer Link 48.

PD-L1 protein expression is defined as the percentage of tumor cells exhibiting positive membrane staining at any intensity.

PD-L1 expression as detected by PD-L1 IHC 28-8 IHC pharmDx in non-squamous NSCLC may be associated with enhanced survival from OPDIVO® (nivolumab).

PD-L1 IHC 28-8 pharmDx Interpretation Manual

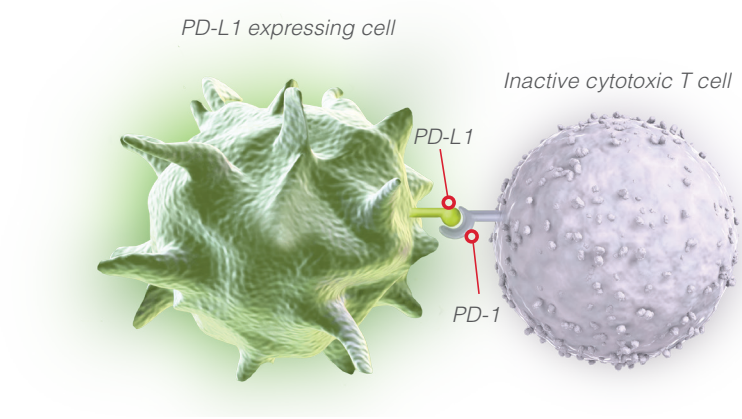
This PD-L1 IHC 28-8 pharmDx Interpretation Manual is provided as a tool to help guide pathologists and laboratory technicians to achieve correct and reproducible results. The goal of this manual is to familiarize you with the requirements for scoring non-squamous NSCLC specimens stained with PD-L1 IHC 28-8 pharmDx. Micrographs of example cases are provided for reference. The PD-L1 IHC 28-8 pharmDx package insert contains guidelines and technical tips for ensuring high-quality staining in your laboratory.

Review of this PD-L1 IHC 28-8 pharmDx Interpretation Manual will provide a solid foundation for evaluating slides stained with PD-L1 IHC 28-8 pharmDx. For more details, please refer to the current version of the package insert provided with PD-L1 IHC 28-8 pharmDx or visit www.dako.com.

The included photomicrographs are non-squamous NSCLC unless otherwise noted.

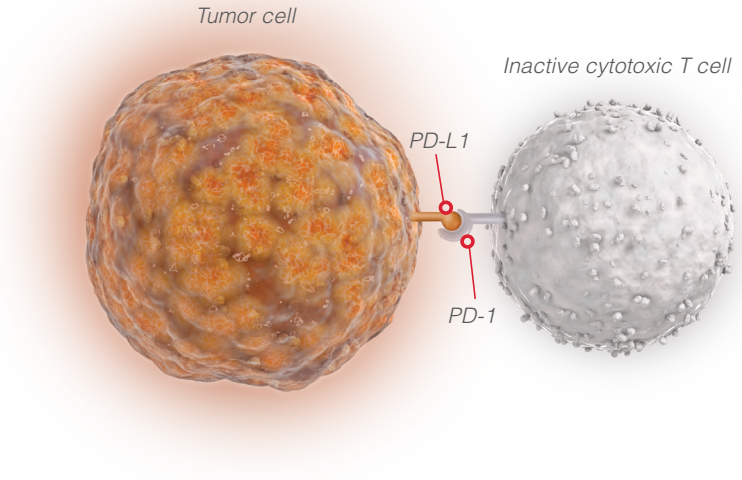
OPDIVO is a registered trademark of Bristol-Myers Squibb Company.

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



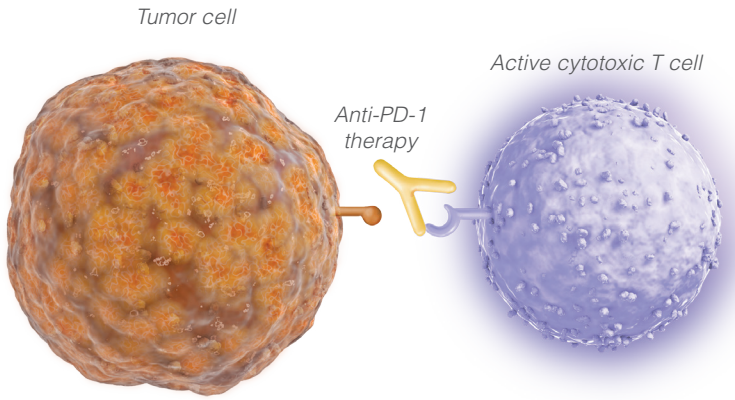
Limiting damage to healthy tissue

Inactivation of T cells limits damage to healthy tissue.



The tumor escapes detection

Inactivation of T cells reduces tumor cell killing.



Immuno-oncology therapies harness the immune response to fight tumors

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

PD-L1 IHC 28-8 pharmDx

Code SK005

PD-L1 IHC 28-8 pharmDx contains optimized reagents and protocol required to complete an IHC staining procedure of FFPE specimens using Autostainer Link 48 and Dako 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, and then are 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 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 formalin-fixed, paraffin-embedded human cell lines are provided to validate staining runs.

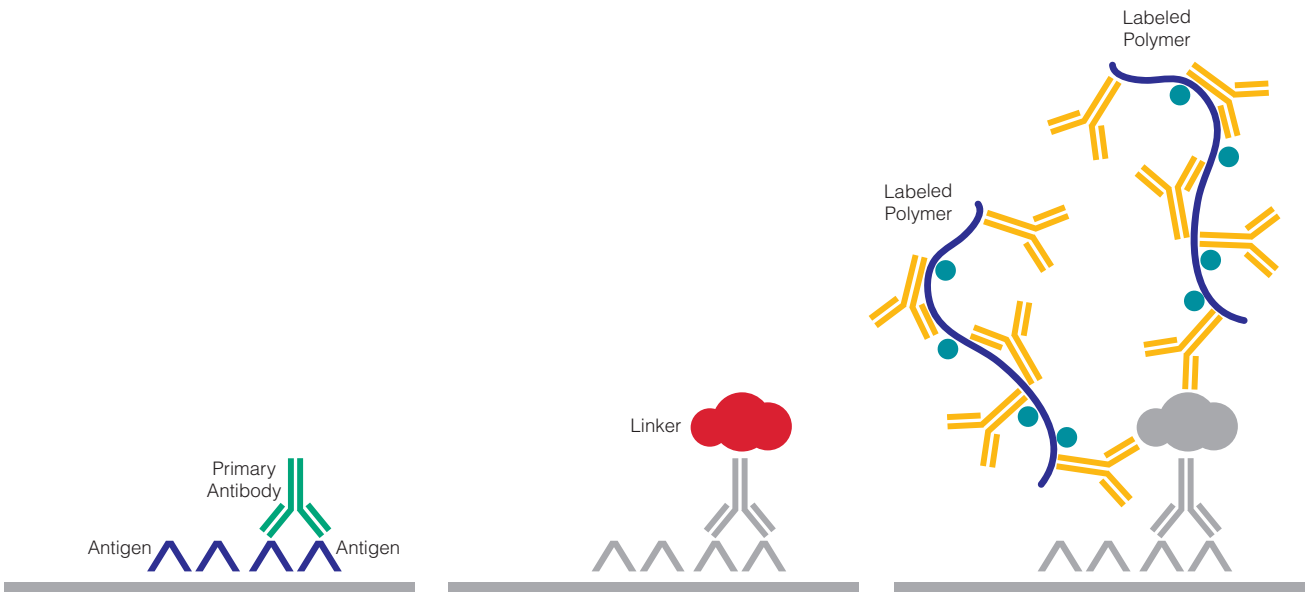


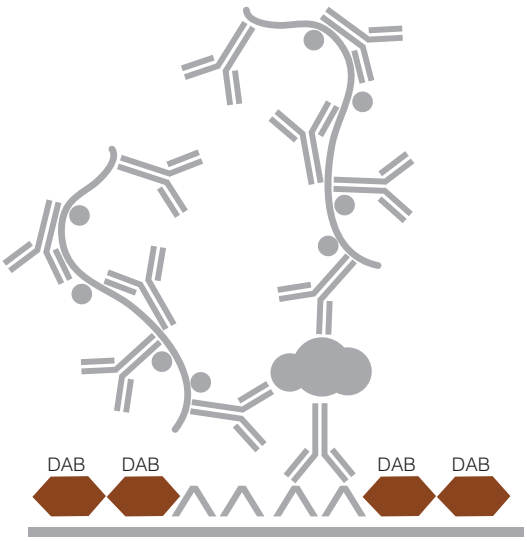
Figure 1: PD-L1 IHC 28-8 pharmDx staining procedure

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

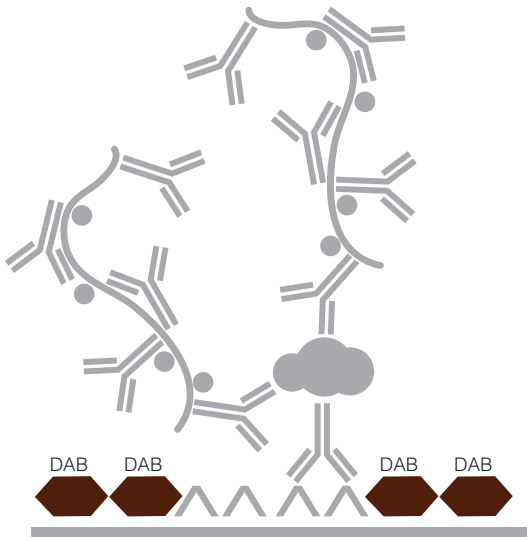
- EnVision FLEX Target Retrieval Solution, Low pH, 50x
- Peroxidase-Blocking Reagent
- Primary Antibody: Monoclonal Rabbit Anti-PD-L1, Clone 28-8
- Negative Control Reagent
- Rabbit LINKER
- Visualization Reagent-HRP
- DAB+ Substrate Buffer
- DAB+ Chromogen
- DAB Enhancer
- PD-L1 IHC 28-8 pharmDx Control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

EnVision FLEX Wash Buffer, 20x, Code K8007, and EnVision FLEX Hematoxylin, Code K8008, are required but not included in the kit. Refer to Instructions for Use for a complete list of required materials and equipment.

Figure 2: PD-L1 IHC 28-8 pharmDx components



Application of DAB+ Substrate Chromogen Solution.



Application of DAB Enhancer.

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

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 thorough understanding of the product instructions by the user.

Specimen Collection and Preparation

Specimens must be handled in a way which preserves the tissue for immunohistochemical staining. Confirm appropriate tumor morphology and the presence of sufficient number of cells for evaluation. Use standard methods of tissue processing for all specimens.

Control Tissue

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 Slides.

Select positive and negative control tissue from fresh non-squamous NSCLC specimens. Fix, process, and embed the control tissue in the same manner as patient specimens. Control tissue processed differently from the patient specimen validates reagent performance only and does not verify tissue preparation. The ideal positive control tissue gives weak to moderate positive staining. The variety of different cell types present in most tissue sections offers internal negative control sites; this should be verified by the user. A suggested non-squamous NSCLC negative control tissue is one that shows no staining in tumor cells but possesses stained normal pulmonary macrophages.

Tissue Processing

Formalin-fixed, paraffin-embedded tissues are suitable 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. An ischemia time from excision to formalin fixation start time of less than 30 minutes followed by immersion in neutral buffered formalin for 24-48 hours is recommended. 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 Fisherbrand Superfrost Plus charged slides. Store tissue sections in the dark at 2-8 °C to preserve antigenicity, and stain within 4 months of sectioning.

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. All of the required steps and incubation times for staining are preprogrammed in the DakoLink software.

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.

Reagent Preparation

Equilibrate all components to room temperature (20-25 °C) prior to immunostaining. Do not use after the expiration date printed on the outside package.

EnVision FLEX Target Retrieval Solution, Low pH
Dilute EnVision FLEX Target Retrieval Solution, Low pH, 50x 1:50 using distilled or deionized water (reagent-quality water). One 30 mL bottle of concentrate provides 1.5 L of working solution which is sufficient to fill one PT Link tank which will treat up to 24 slides per use. The pH of the working solution should be 6.1 ± 0.2. Discard EnVision FLEX Target Retrieval Solution, Low pH after three uses and do not use after 5 days following dilution.

EnVision FLEX Wash Buffer, Code K8007
Dilute EnVision FLEX Wash Buffer, 20x 1:20 using distilled or deionized water (reagent-quality water) for the wash steps. Store unused 1x buffer at 2-8 °C for no more than one month. Discard if cloudy in appearance.

DAB+ Substrate-Chromogen Solution
Add 1 drop of DAB+ Chromogen per mL of DAB+ Substrate Buffer and mix. Prepared DAB+ Substrate-Chromogen is stable for 5 days if stored in the dark at 2-8 °C. Mix the DAB+ Substrate-Chromogen Solution thoroughly prior to use. Any precipitate developing in the solution 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 useable volume and does not account for the “dead volume” of DAB+ Substrate Buffer in the bottle.

- The color of the DAB+ Chromogen may vary from clear to lavender brown. This will not affect the performance of the product. Dilute per the guidelines above. Adding excess DAB+ Chromogen to the DAB+ Substrate Buffer results in deterioration of the positive signal.

Controls to Assess Staining Quality

Include one PD-L1 IHC 28-8 pharmDx Control Slide stained with the Primary Antibody in each staining run. For each set of test conditions, include positive and negative control tissue stained with the Primary Antibody and Negative Control Reagent in each staining run. Use the Negative Control Reagent in addition to the Primary Antibody on a sequential section of each patient specimen.

Deparaffinization, Rehydration and Target Retrieval

Use PT Link, Code PT100, to perform the Deparaffinization, Rehydration and Target retrieval 3-in-1 procedure.

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

Staining and Counterstaining

Place the Autostainer rack with slides on the Autostainer Link 48. Ensure slides remain wet with buffer while loading and prior to initiating the run. Dried tissue sections may display increased nonspecific staining.

Select the PD-L1 IHC 28-8 pharmDx protocol. 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 is included in the staining protocol.

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
Regular preventive maintenance is performed on the Autostainer Link 48 and PT Link?	☐	☐
PD-L1 IHC 28-8 pharmDx is used before the expiration date printed on the outside of the box?	☐	☐
All PD-L1 IHC 28-8 pharmDx components, including Control Slides, are stored in the dark at 2-8 °C?	☐	☐
All PD-L1 IHC 28-8 pharmDx components, including Control Slides, are equilibrated to room temperature (20-25 °C) prior to immunostaining?	☐	☐
Appropriate positive and negative control tissue from non-squamous NSCLC are identified?	☐	☐
Tissues are fixed in neutral buffered formalin?	☐	☐
Tissues are infiltrated with melted paraffin, at or below 60 °C?	☐	☐
Tissue sections of 4-5 µm are mounted on FLEX IHC Microscope Slides or Fisherbrand Superfrost Plus charged slides?	☐	☐
Specimens are stained within 4 months of sectioning when stored in the dark at 2-8 °C?	☐	☐
EnVision FLEX Target Retrieval Solution, Low pH is prepared properly?	☐	☐
EnVision FLEX Wash Buffer is prepared properly?	☐	☐
DAB+ Substrate-Chromogen Solution is prepared properly?	☐	☐
The Deparaffinization, Rehydration and Target Retrieval 3-in-1 procedure is followed, using PT Link?	☐	☐
Slides remain wet with buffer while loading and prior to initiating run on Autostainer Link 48?	☐	☐
The PD-L1 IHC 28-8 pharmDx protocol is selected on Autostainer Link 48?	☐	☐
Slides are counterstained with EnVision FLEX Hematoxylin?	☐	☐
Do you have all the necessary equipment to perform the PD-L1 IHC 28-8 pharmDx according to protocol? If not, specify what is missing in 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

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 to assess staining quality. 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 slide stained with the Negative Control Reagent for each patient case. Lastly, examine the patient specimen stained with Primary Antibody to assess staining of viable tumor cells.

PD-L1 staining is defined as complete circumferential or partial linear plasma membrane staining at any intensity.

Cytoplasmic staining, if present, is not considered positive for scoring purposes. Non-malignant cells and immune cells (e.g., such as infiltrating lymphocytes or macrophages) may also stain with PD-L1; however, these should not be included in the scoring for the 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.

1. **Patient Specimen stained with H&E**
   Histology and preservation quality
   Acceptable

2. **Control Slide**
   Stained with PD-L1 Primary Antibody
   Acceptable

3. **Positive Control Tissue Slides**
   Stained with PD-L1
   Primary Antibody and NCR
   Acceptable

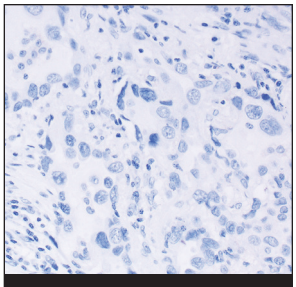
4. **Negative Control Tissue Slides**
   Stained with PD-L1
   Primary Antibody and NCR
   Acceptable

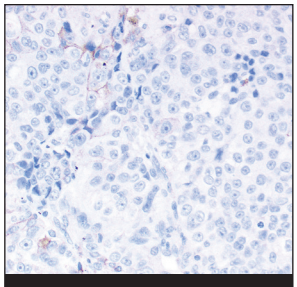
5. **Patient Specimen stained with NCR**
   Acceptable

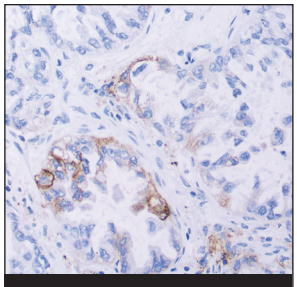
6. **Patient Specimen stained with PD-L1 Primary Antibody**
   > 100 Viable Tumor Cells
   PD-L1 Scoring

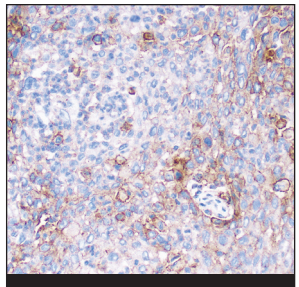
Exclude from Scoring
- Cytoplasmic staining
- Immune cells
- Normal cells
- Necrotic cells

Score viable tumor cells exhibiting complete circumferential or partial linear plasma membrane staining at any intensity. Determine the percentage of stained viable tumor cells in the entire specimen.


PD-L1 expression < 1%


PD-L1 expression ≥ 1%


PD-L1 expression ≥ 5%


PD-L1 expression ≥ 10%

1 Patient Specimen Stained with H&E

A hematoxylin and eosin (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 serial sections from the same paraffin block of the specimen.

2 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 interpretation of patient results.

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

- At least 80% of the cells contain plasma membrane staining of at least 2+ average staining intensity
- Any background staining is of less than 1+ staining intensity

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

- No plasma membrane staining
- Any background staining is of less than 1+ staining intensity

Note: 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, or cytoplasmic staining with ≥ 1 + intensity within the boundaries of the cell pellet are acceptable.

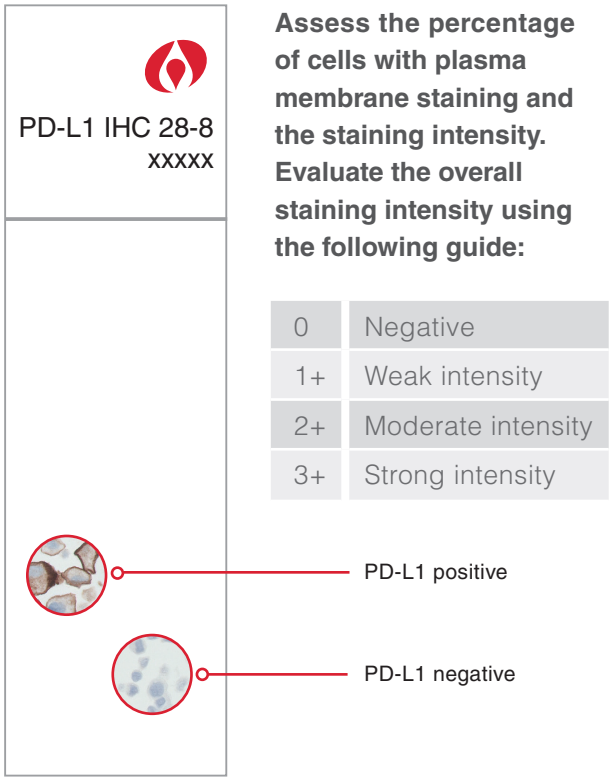
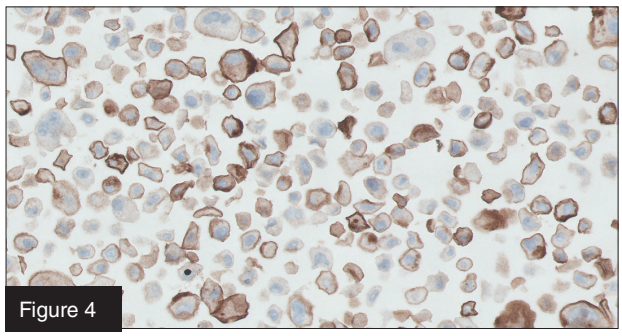


Figure 3: Each Control Slide contains sections of cell pellets with positive and negative PD-L1 expression.



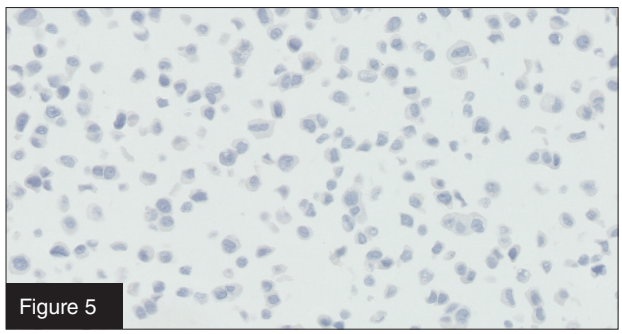


Figure 4: Acceptable staining of positive pellet.

Figure 5: Acceptable staining of negative pellet.

3 Positive Control Tissue Slides

Examine the positive non-squamous NSCLC control tissue to ascertain that tissues are correctly prepared and reagents are functioning properly. Any background staining should be of less than 1+ staining intensity. Exclude necrotic or degenerated malignant cells from evaluation. If staining of positive control tissues is not satisfactory, all results with the patient specimens should be considered invalid.

4 Negative Control Tissue Slides

Examine the negative non-squamous NSCLC control tissue to ascertain no unintended staining. Any background staining should be of less than 1+ staining intensity. If plasma membrane staining of malignant 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 interpretation of patient results.

5 Patient Specimen Stained with Negative Control Reagent

Examine the patient specimen stained with Negative Control Reagent to ascertain that reagents are functioning properly. Absence of plasma membrane staining of viable tumor cells is satisfactory. See Figure 6 for a satisfactory example. If any staining is not satisfactory, results with the patient specimen should be considered invalid.

The Negative Control Reagent indicates non-specific background staining and allows better interpretation of patient specimen stained with the Primary Antibody.

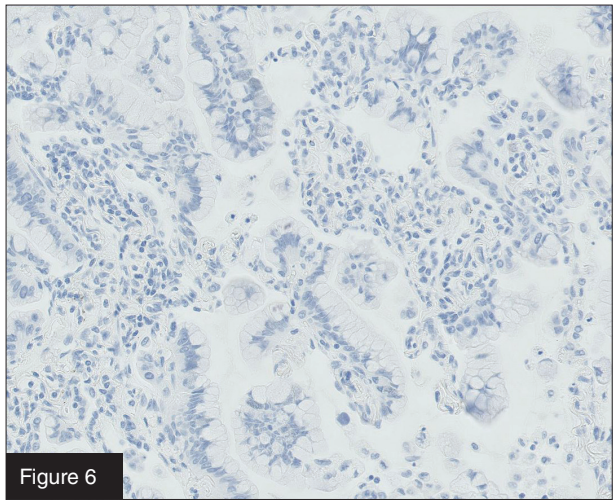


Figure 6: Absence of plasma membrane staining in non-squamous NSCLC stained with Negative Control Reagent.

6 Patient Specimen Stained with Primary Antibody

Staining should be assessed within the context of any non-specific background staining of the patient specimen stained with Negative Control Reagent. A minimum of 100 viable tumor cells should be present in the PD-L1 stained patient slide to determine the percentage of stained cells.

1	At 4x objective magnification, carefully examine the tumor areas of the entire specimen. Well-preserved and well-stained areas of the specimen should be used to evaluate PD-L1 staining.
2	At 10-40x objective magnification, score viable tumor cells exhibiting complete circumferential or partial linear plasma membrane staining at any intensity. Exclude cytoplasmic staining from scoring. Exclude immune cells, normal cells, and necrotic cells from scoring.
3	At 10-40x objective magnification, determine the percentage of stained viable tumor cells in the entire specimen.

See Figures 7-10 for examples of non-squamous NSCLC stained with Primary Antibody.

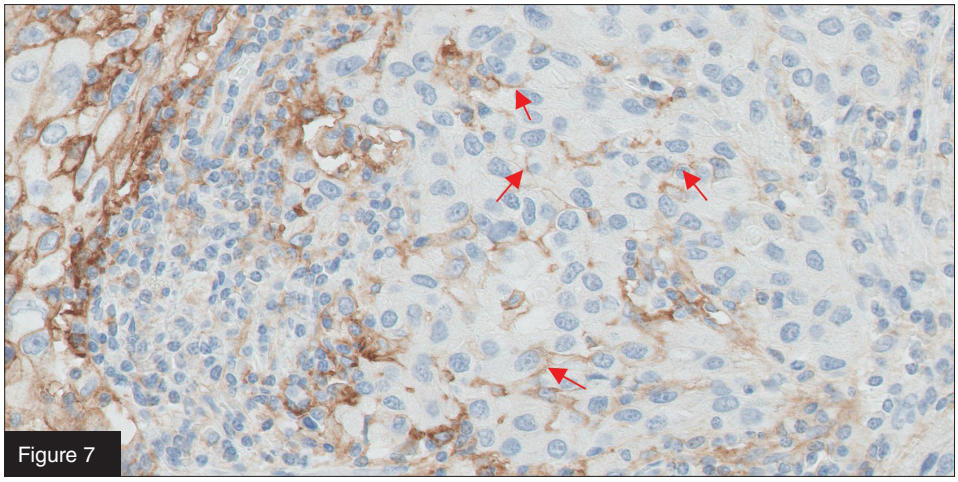
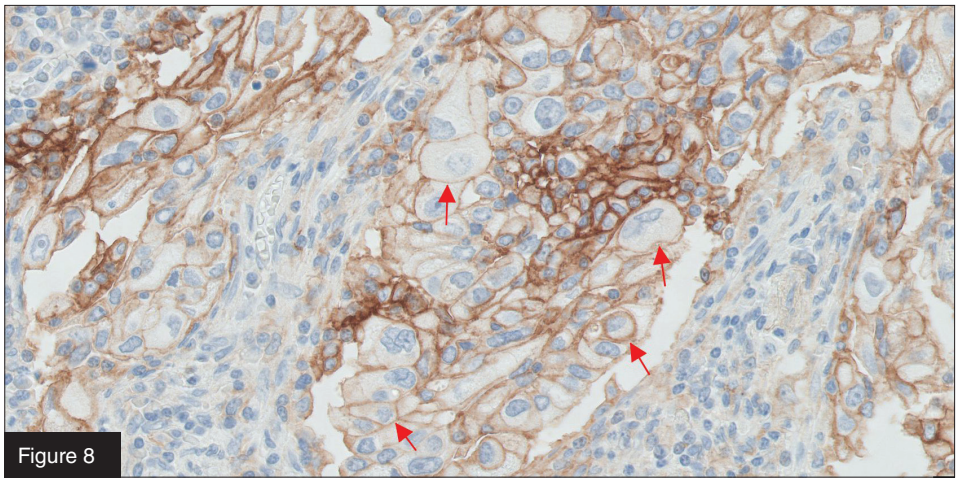
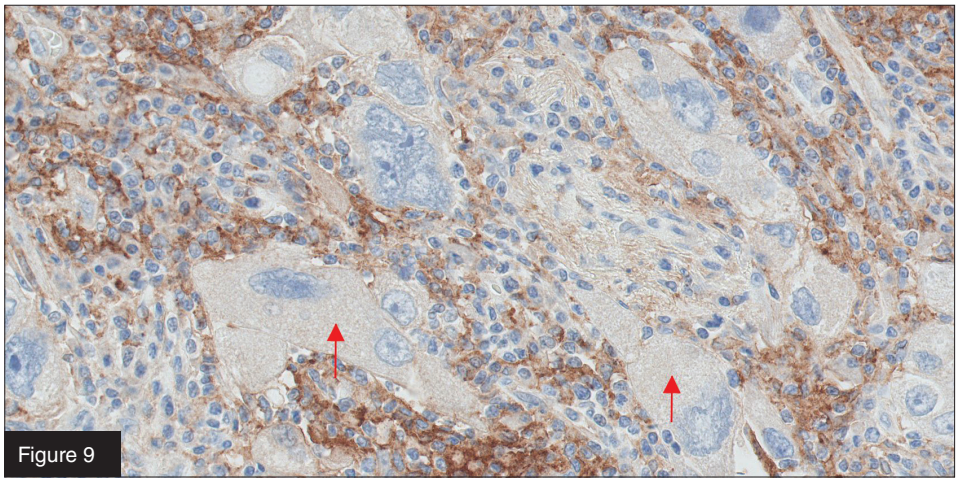


Figure 7: Red arrows show partial linear plasma membrane staining of viable tumor cells.





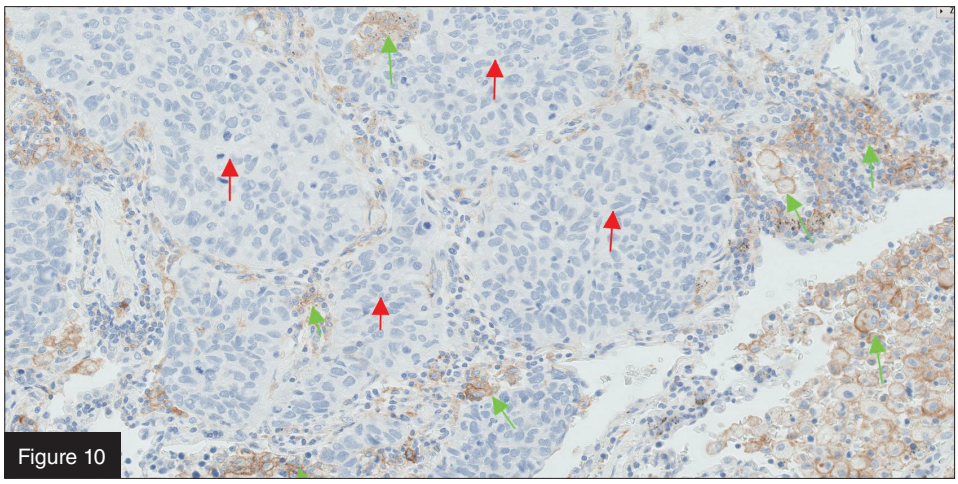


Figure 8: Red arrows show complete circumferential plasma membrane staining of viable tumor cells.

Figure 9: Red arrows show cytoplasmic staining as observed throughout the specimen. Exclude cytoplasmic staining from scoring.

Figure 10: Red arrows show viable tumor cells. Green arrows show staining of immune cells. Exclude immune cells from scoring.

Guidelines for Scoring PD-L1 IHC 28-8 pharmDx

Dako emphasizes that scoring of PD-L1 IHC 28-8 pharmDx must be performed in accordance with the guidelines established in the package insert and within the context of best practices and the pathologist's experience and best medical judgment.

The percentage of viable tumor cells exhibiting positive membrane staining at any intensity in the entire specimen determines the PD-L1 IHC 28-8 pharmDx result. Scoring guidelines and reporting recommendations are presented in Table 1.

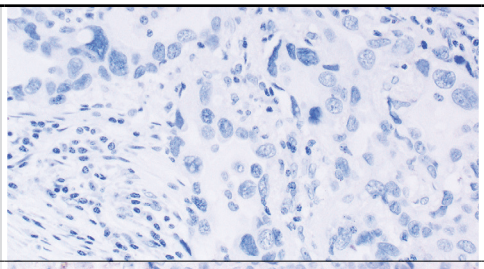
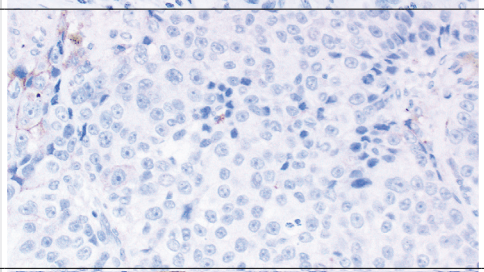
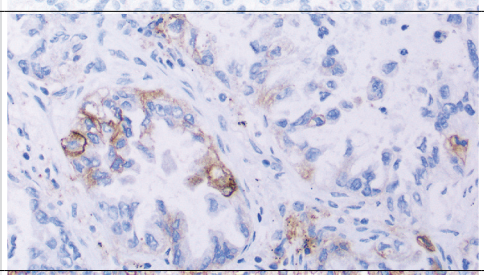
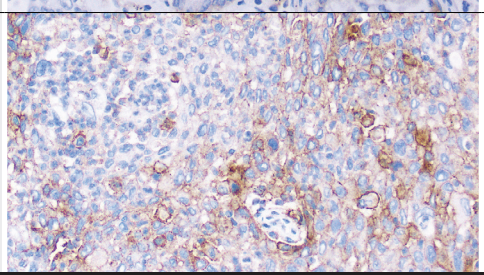
Staining Pattern	Examples of non-squamous NSCLC	Examples of result reporting
< 1% of the viable tumor cells exhibit complete circumferential or partial linear plasma membrane staining at any intensity.		PD-L1 expression < 1%
≥ 1% of the viable tumor cells exhibit complete circumferential or partial linear plasma membrane staining at any intensity.		PD-L1 expression ≥ 1%
≥ 5% of the viable tumor cells exhibit complete circumferential or partial linear plasma membrane staining at any intensity.		PD-L1 expression ≥ 5%
≥ 10% of the viable tumor cells exhibit complete circumferential or partial linear plasma membrane staining at any intensity.		PD-L1 expression ≥ 10%

Table 1: Guidelines for scoring and reporting of PD-L1 IHC 28-8 pharmDx

Clinical Interpretation of PD-L1 IHC 28-8 pharmDx Results

PD-L1 IHC 28-8 pharmDx may be used to associate PD-L1 expression with enhanced survival from OPDIVO in non-squamous NSCLC patients.

Clinical utility of PD-L1 IHC 28-8 pharmDx was evaluated in CheckMate -057 (CA209057), a phase 3, randomized, open-label study of nivolumab vs. docetaxel in adult (> 18 years) patients with advanced or metastatic non-squamous NSCLC after failure of prior platinum doublet-based chemotherapy. Subjects were randomized 1:1 and stratified according to the following factors: prior use of maintenance therapy vs. no use of maintenance therapy and second-line vs. third-line therapy (to account for prior tyrosine kinase inhibitor use). Pre-study (baseline) tumor tissue specimens were collected prior to randomization and prior to first treatment to conduct pre-planned analyses of efficacy according to predefined baseline PD-L1 expression levels (secondary objective). The primary endpoint was overall survival (OS). Other secondary endpoints were objective response rate, progression-free survival, and disease-related symptom improvement by 12 weeks, as measured by the Lung Symptom Cancer Scale.

In CA209057, patients with PD-L1 expression by all predefined expression levels in the OPDIVO group were associated with enhanced survival compared to docetaxel, whereas survival was similar to docetaxel in patients with no PD-L1 expression. Meaningful differences in median OS were observed in nivolumab over docetaxel subgroups when analyzed by PD-L1 expression level. Median OS was 17.1, 18.2, and 19.4 months for nivolumab subjects compared to 9.0, 8.1, and 8.0 months for docetaxel subjects with ≥ 1%, ≥ 5%, and ≥ 10% PD-L1 expression levels, respectively. There were no differences in OS between the treatment groups in subjects with < 1%, < 5%, and < 10% expression levels, with ranges of median OS of 9.7 to 10.4 months for nivolumab and 10.1 to 10.3 months for docetaxel.

Examples of PD-L1 IHC 28-8 pharmDx Immunostaining

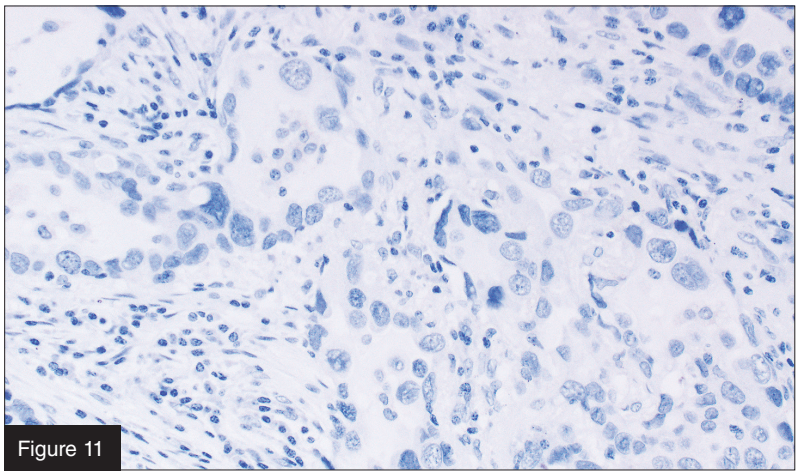


Figure 11

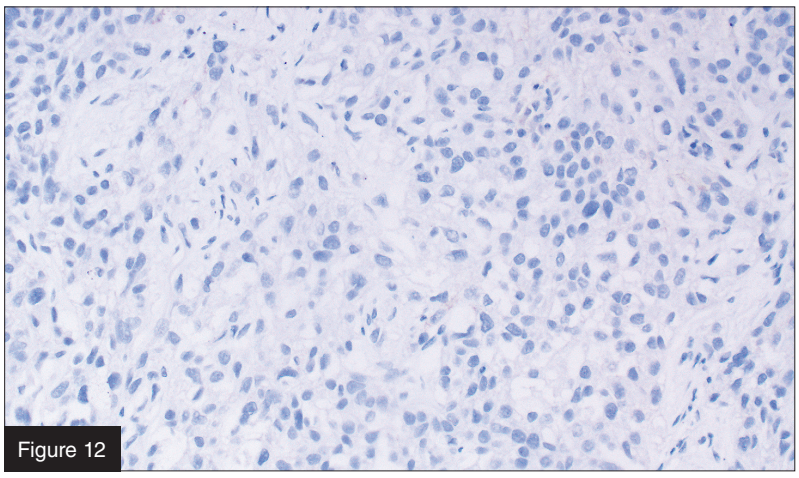


Figure 12

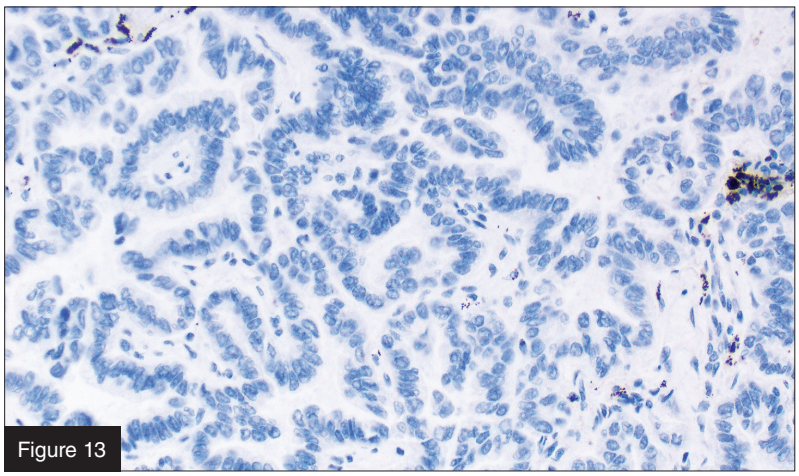


Figure 13

Figure 11: PD-L1 expression < 1%. 20x objective magnification.

Figure 12: PD-L1 expression < 1%. 20x objective magnification.

Figure 13: PD-L1 expression < 1%. 20x objective magnification.

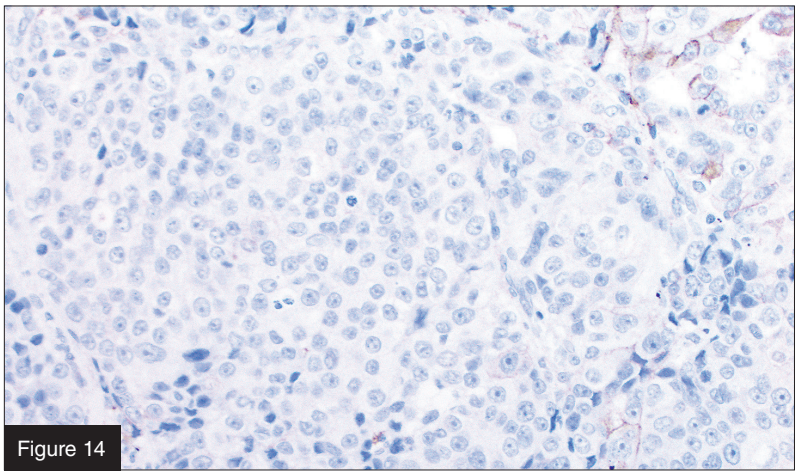


Figure 14

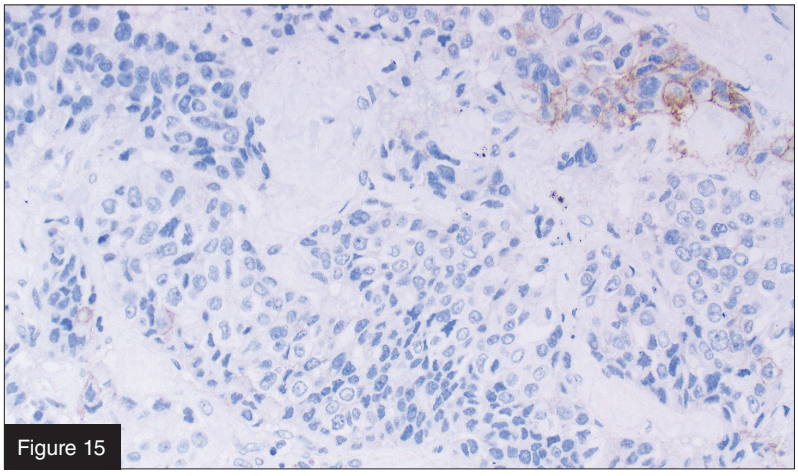


Figure 15

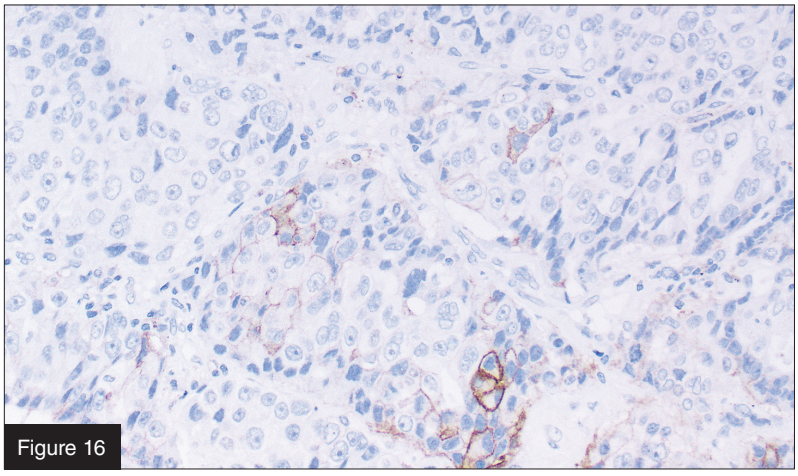


Figure 16

Figure 14: PD-L1 expression ≥ 1%. 20x objective magnification.

Figure 15: PD-L1 expression ≥ 1%. 20x objective magnification.

Figure 16: PD-L1 expression ≥ 1%. 20x objective magnification.

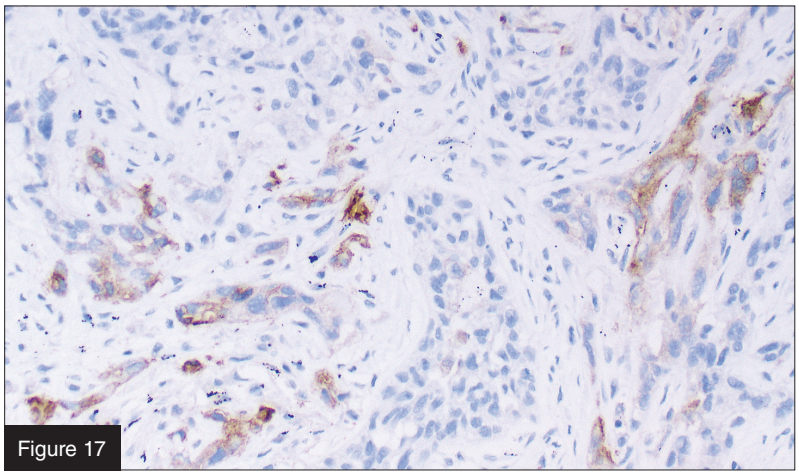
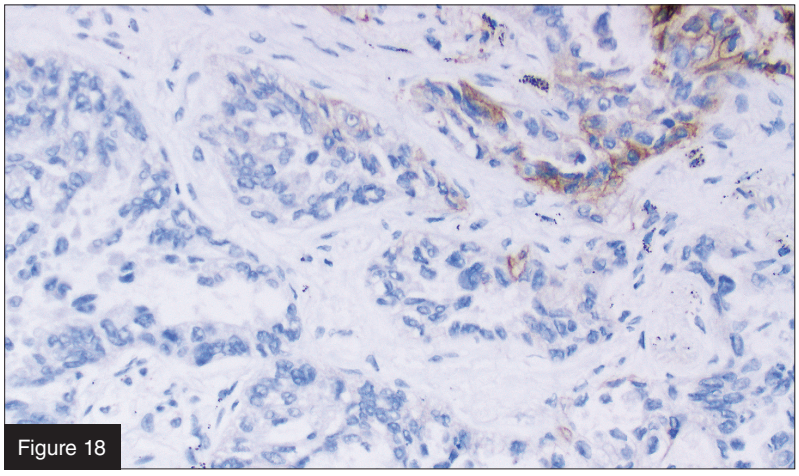


Figure 17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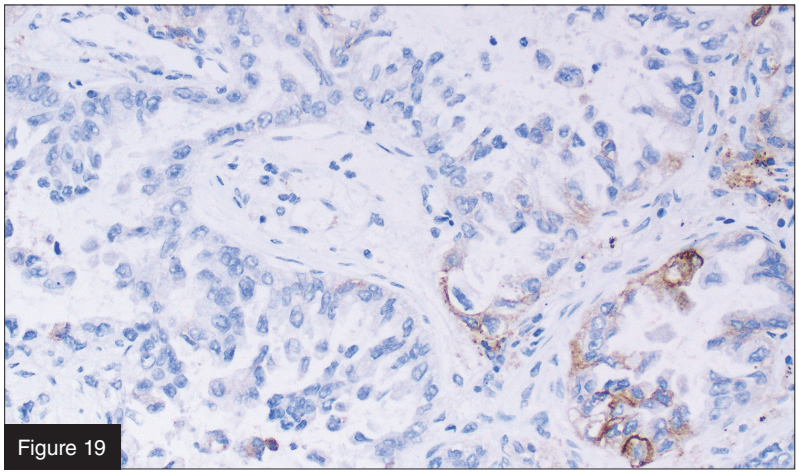
Figure 19

Figure 17: PD-L1 expression ≥ 5%. 20x objective magnification.

Figure 18: PD-L1 expression ≥ 5%. 20x objective magnification.

Figure 19: PD-L1 expression ≥ 5%. 20x objective magnification.

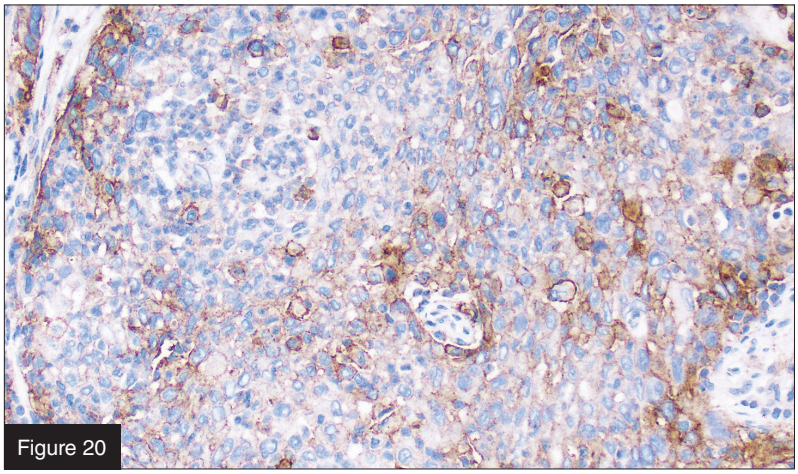

Figure 20

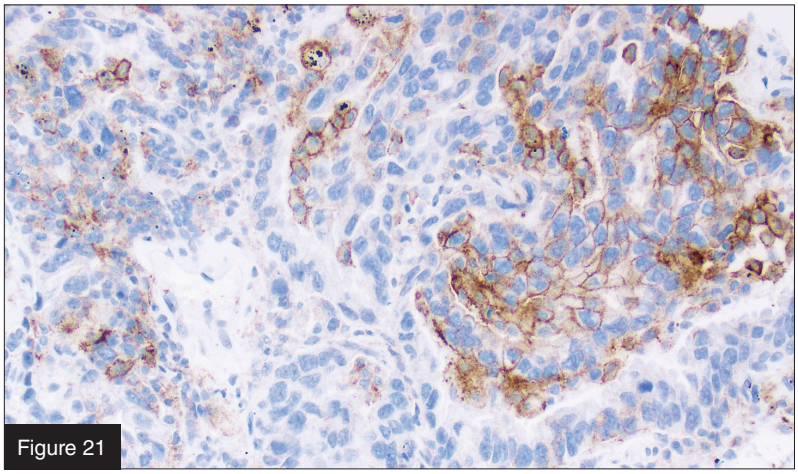

Figure 21

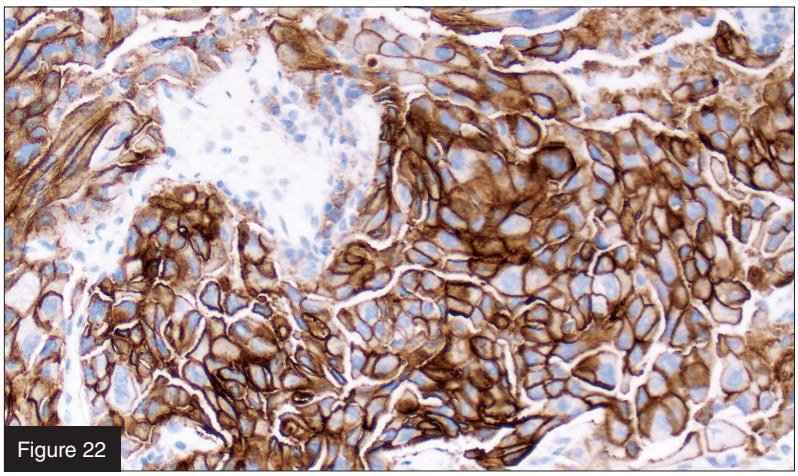

Figure 22

Figure 20: PD-L1 expression ≥ 10%. 20x objective magnification.

Figure 21: PD-L1 expression ≥ 10%. 20x objective magnification.

Figure 22: PD-L1 expression ≥ 10%. 20x objective magnification.

Troubleshooting Guide for PD-L1 IHC 28-8 pharmDx

Problem	Probable Cause	Suggested Action
1. No staining of slides.	1a. Programming error.	1a. Verify that the SK005 PD-L1 IHC 28-8 pharmDx protocol was selected for programming of slides.
	1b. Lack of reaction with DAB+ Substrate-Chromogen Solution.	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.
2a. Weak staining of specimen slides.	2a. Inappropriate fixation method used.	2a. Ensure that only approved fixatives and fixation methods are used.
2b. Weak staining of specimen slides or of the positive cell line on the Dako-provided Control Slide.	2b. Inadequate target retrieval.	2b. Verify that the 3-in-1 pre-treatment procedure was correctly performed.
3. Excessive background staining of slides.	3a. Paraffin incompletely removed.	3a. Verify that the 3-in-1 pre-treatment procedure was correctly performed.
	3b. Slides dried while loading onto the Autostainer Link 48.	3b. Ensure slides remain wet with buffer while loading and prior to initiating run.
	3c. Nonspecific binding of reagents to tissue section.	3c. Check for proper fixation of the specimen and/or the presence of necrosis.
4. Tissue detached from slides.	4a. Use of incorrect microscope slides.	4a. Use Dako FLEX IHC Microscope Slides, Code K8020, or Fisherbrand Superfrost Plus charged slides.
	4b. Inadequate preparation of specimens.	4b. Cut sections should be placed in a 58 ± 2 °C oven for one hour prior to staining.
5. Excessively strong specific staining.	5a. Inappropriate fixation method used.	5a. Ensure that only approved fixatives and fixation methods are used.
	5b. Inappropriate wash buffer used.	5b. Use only Dako Wash Buffer, Code K8007.
6. The Target Retrieval Solution is cloudy in appearance when heated.	6. Components in the Target Retrieval Solution cause the reagent to appear cloudy when heated.	6. No action required. This is normal and does not affect staining.

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