Your Guide for Accurate Scoring in Triple-Negative Breast Cancer (TNBC) Using PD-L1 IHC 22C3 pharmDx (SK006)

Agilent Dako

Use this quick scoring guide as a reference when evaluating TNBC specimens for PD-L1 expression using PD-L1 IHC 22C3 pharmDx.

For more information on Combined Positive Score (CPS) assessment, review the TNBC Interpretation Manual at www.agilent.com.

Steps for scoring

Determine specimen adequacy
Verify that the specimen has ≥ 100 viable tumor cells

Evaluate controls
Ensure that Control Cell Line Slide and both lab-supplied and patient tissue controls demonstrate acceptable staining

Evaluate PD-L1 staining
Evaluate the number of PD-L1 staining cells (CPS numerator) and the total number of viable tumor cells (CPS denominator)

Calculate CPS to determine PD-L1 expression level
Report numerical CPS and PD-L1 expression level:*
CPS < 10
or **CPS ≥ 10**

** Refer to the Interpretation Manual for suggested information to include when reporting results for PD-L1 IHC 22C3 pharmDx*

Definition of CPS and PD-L1 staining cells

CPS is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100.

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

Note: *CPS is reported as a whole number. Although the result of the calculation can exceed 100, the maximum score is defined as CPS 100.*

By definition, **PD-L1 staining cells** in TNBC are:

- **Viable invasive tumor cells** with perceptible and convincing partial or complete linear membrane staining (at any intensity) that is perceived distinct from cytoplasmic staining at 20× magnification (Figures 3 and 4)
- **Lymphocytes and macrophages** (mononuclear inflammatory cells, MICs) within the tumor nests and/or adjacent supporting stroma with membrane and/or cytoplasmic staining (at any intensity). MICs must be directly associated with the response against the tumor (Figures 5 and 6)

CPS < 10

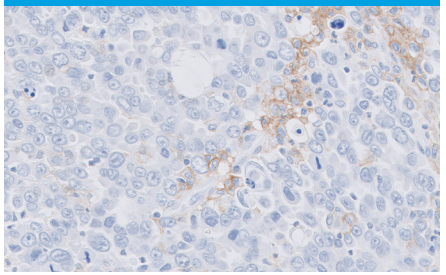

Figure 1: TNBC specimen stained with PD-L1 IHC 22C3 pharmDx primary antibody exhibiting a CPS of 6, however any numerical CPS between 4–8 could be assigned to this image (20× magnification).

CPS ≥ 10

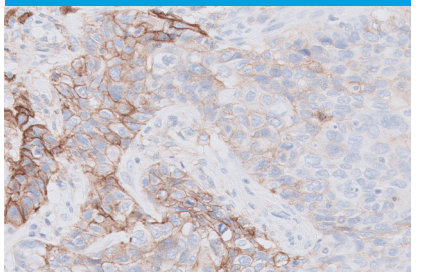

Figure 2: TNBC specimen stained with PD-L1 IHC 22C3 pharmDx primary antibody exhibiting a CPS of 60, however any numerical CPS between 57–63 could be assigned to this image (20× magnification).

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

Some data and biospecimens used in this project were provided by Centre Antoine Lacassagne (CAL; Nice, France) with appropriate ethics approval and through Trans-Hit Biomarkers Inc.

Intended Use

For in vitro diagnostic use.

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

Triple-Negative Breast Cancer (TNBC)

PD-L1 protein expression in TNBC is determined by using Combined Positive Score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. The specimen should be considered to have PD-L1 expression if CPS ≥ 10.

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

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

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TNBC CPS numerator

Tissue Elements	Included	Excluded
Tumor Cells	Convincing partial or complete linear membrane staining (at any intensity) of viable invasive tumor cells	– Non-staining tumor cells – Tumor cells with only cytoplasmic staining – Carcinoma in situ (DCIS and LCIS)
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 directly associated with the response to the tumor are scored	– Non-staining MICs – MICs associated with DCIS and LCIS – MICs associated with benign structures – MICs (including lymphoid aggregates) not directly associated with the response to the tumor – Neutrophils, eosinophils, and plasma cells
Other Cells	Not included	– Benign epithelial cells – Stromal cells (including fibroblasts) – Necrotic cells and/or cellular debris

TNBC CPS denominator

Included	Excluded
All viable invasive tumor cells	– Non-viable tumor cells – Carcinoma in situ (DCIS and LCIS)
Not included	All immune cells
Not included	– Benign cells – Stromal cells (including fibroblasts) – Necrotic cells and/or cellular debris

** In MICs, membrane and cytoplasmic staining are often indistinguishable due to high nuclear to cytoplasmic ratio. Therefore, membrane and/or cytoplasmic staining of MICs are included in the score; † Adjacent MICs are defined as being within the same 20× field as the tumor. However, MICs that are NOT directly associated with the response against the tumor should be excluded; ‡ Macrophages and histiocytes are considered the same cells*

Partial linear membrane staining

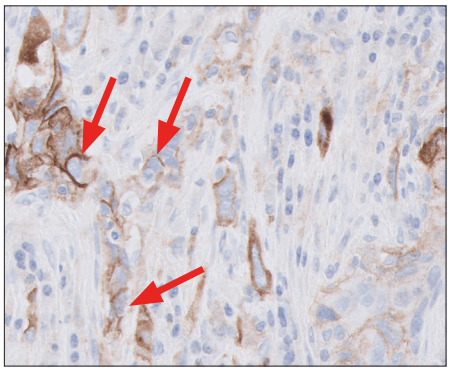

Figure 3: TNBC specimen stained with PD-L1 IHC 22C3 pharmDx primary antibody exhibiting partial linear membrane staining of tumor cells (arrows) (20× magnification).

Complete linear membrane staining

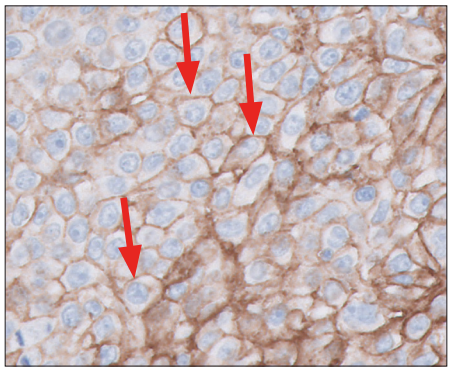

Figure 4: TNBC specimen stained with PD-L1 IHC 22C3 pharmDx primary antibody exhibiting complete linear membrane staining of tumor cells (arrows) (20× magnification).

Tumor-associated immune cells

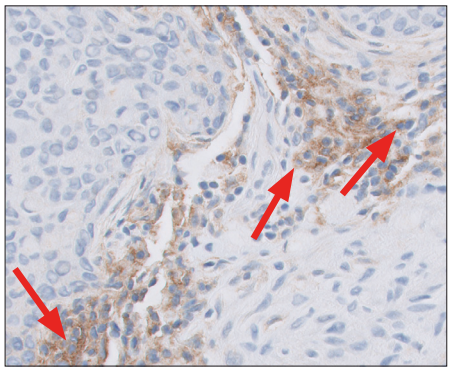

Figure 5: When positioning the edge of the tumor mass in the approximate center of a 20× field, PD-L1 staining MICs (arrows) that are present within the same field should be included in the numerator (20× magnification).

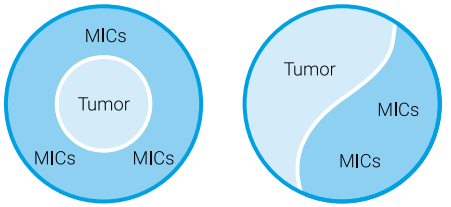

Figure 6: Simulation of a 20× microscope field showing tumor surrounded by PD-L1 staining tumor-associated MICs that should be included in the numerator.

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

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This information is subject to change without notice.

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