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

For more information on Combined Positive Score (CPS) calculation, review the ESCC Interpretation Manual.

Steps for scoring

<table>
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<tr>
<th>Determine specimen adequacy</th>
<th>Evaluate controls</th>
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<td>Verify that the specimen has ≥ 100 viable tumor cells</td>
<td>Ensure that Control Cell Line Slide and lab-supplied and patient tissue controls demonstrate acceptable staining</td>
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<table>
<thead>
<tr>
<th>Evaluate PD-L1 staining</th>
<th>Calculate CPS to determine PD-L1 expression level</th>
</tr>
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<tr>
<td>Estimate the number of PD-L1 staining cells (CPS numerator) and the total number of viable tumor cells (CPS denominator)</td>
<td>Report numerical CPS and PD-L1 expression level: CPS &lt; 10 or CPS ≥ 10</td>
</tr>
</tbody>
</table>

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.

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

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

- **Viable tumor cells** with perceptible and convincing partial or complete linear membrane staining (at any intensity) that is perceived distinct from cytoplasmic staining

- **Lymphocytes and macrophages** (mononuclear inflammatory cells, MICs) within the tumor nests and/or adjacent supporting stroma with membrane and/or cytoplasmic staining (at any intensity). MICs must be directly associated with the response against the tumor

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.

**Figure 1:** ESCC specimen stained with PD-L1 antibody exhibiting a CPS of 4, however any numerical CPS between 2–6 could be assigned to this image (20× magnification).

**Figure 2:** ESCC specimen stained with PD-L1 antibody exhibiting a CPS of 45, however any numerical CPS between 40–50 could be assigned to this image (20× magnification).

**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) esophageal squamous cell carcinoma (ESCC) tissue using EnVision FLEX visualization system on Autostainer Link 48.

**Esophageal Squamous Cell Carcinoma (ESCC)**

PD-L1 protein expression in ESCC 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 ESCC patients for treatment with KEYTRUDA® (pembrolizumab). See the KEYTRUDA® product label for specific clinical circumstances guiding PD-L1 testing.

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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Tumor-associated immune cells

Figure 4: When positioning the edge of a 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.

Data and biospecimens used in this project were provided by SageBio LLC, Sharon, MA, USA and by US Biolab, Rockville, MD, with appropriate ethics approval and through Trans-Hit Biomarkers Inc.

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