## Intended Use

For In Vitro Diagnostic Use.
This antibody is intended for use in Immunohistochemical applications on formalinfixed paraffin-embedded tissues (FFPE), frozen tissue sections and cell preparations. Interpretation of results should be performed by a qualified medical professional.

## Immunogen

Synthetic peptide corresponding to N -terminus residues of the human PD-L1 protein.

## Summary and Explanation

Programmed death-ligand 1 (PD-L1) also known as CD274 or B7 homolog 1 (B7-H1) is a protein that in humans is encoded by the CD274 gene. Programmed death-ligand 1 (PD-L1) is a 40 kDa type 1 transmembrane protein that has been speculated to play a major role in suppressing the immune system during particular events such as pregnancy, tissue allografts, autoimmune disease and other diseases.

The upregulation of PD-L1 may allow cancers to evade the host immune system. An analysis of tumor specimens from patients with renal cell carcinoma found that high tumor expression of PD-L1 was associated with increased tumor aggressiveness and a 4.5 -fold increased risk of death. Ovarian cancer patients with higher expression of PDL1 had a significantly poorer prognosis than those with lower expression. PD-L1 expression correlated inversely with intraepithelial CD8+ T-lymphocyte count, suggesting that PD-L1 on tumor cells may suppress antitumor CD8+ $T$ cells. The PD-1/PD-L1 interaction is implicated in autoimmunity from several lines of evidence. In humans, PD-L1 was found to have altered expression in pediatric patients with Systemic lupus erythematosus. Studying isolated PBMC from healthy children, immature myeloid dendritic cells and monocytes expressed little PD-L1 at initial isolation, but spontaneously up-regulated PD-L1 by 24 hours. In contrast, both mDC and monocytes from patients with active SLE failed to upregulate PD-L1 over a 5 -day time course, expressing this protein only during disease remissions.

| Antibody Type | Rabbit Monoclonal | Clone | RBT-PDL1 |
| :--- | :---: | :--- | :---: |
| Isotype | IgG | Reactivity | Paraffin, Frozen |
| Localization | Membranous | Control | Thymus, Tonsil, <br> Placenta, <br> Lymphoblastic <br> Lymphoma, <br> Hodgkin's <br> Lymphoma |
| Species Reactivity |  |  |  | Human $\quad$| ( |
| :--- |

## Presentation

PD-L1 is a rabbit monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

| Catalog No. | Antibody Type | Dilution | Volume/Qty |
| :---: | :---: | :---: | :---: |
| BSB 2649 | Tinto Prediluted | Ready-to-Use | 3.0 mL |
| BSB 2650 | Tinto Prediluted | Ready-to-Use | 7.0 mL |
| BSB 2651 | Tinto Prediluted | Ready-to-Use | 15.0 mL |
| BSB 2652 | Concentrated | $1: 25-1: 100$ | 0.1 mL |
| BSB 2653 | Concentrated | $1: 25-1: 100$ | 0.5 mL |
| BSB 2654 | Concentrated | $1: 25-1: 100$ | 1.0 mL |

## Control Slides Available

| Catalog No. | Quantity |
| :---: | :---: |
| BSB 2655 | 5 slides |

## Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains $<0.1 \%$ sodium azide $\left(\mathrm{NaN}_{3}\right)$ as a preservative. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as laboratory coat, goggles and gloves when handling reagents.
4. Dispose of unused solution with copious amount of water.
5. Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
6. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
7. Follow safety precautions of the heating device used for epitope retrieval
(TintoRetriever Pressure Cooker or similar).
8. For additional safety information refer to Safety Data Sheet for this product.
9. For complete recommendations for handling biological specimens, please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories" (see References in this document).

## Storage Store at $2-8^{\circ} \mathrm{C}$

## Stability

This product is stable up to the expiration date on the product label. Do not use after expiration date listed on package label. Temperature fluctuations should be avoided. Store appropriately when not in use, and avoid prolonged exposure to room temperature conditions.

## Specimen Preparation

Paraffin sections: The antibody can be used on formalin-fixed paraffin-embedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation for best results. Pre-treatment of tissues with heat-induced epitope retrieval (HIER) is recommended using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023), ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033) or ImmunoDNA Digestor (BSB 0108-0112). See reverse side for complete protocol. Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 \& BSB 0042).
Frozen sections and cell preparations: The antibody can be used for labeling acetone-fixed frozen sections and acetone-fixed cell preparations.

## Staining Procedure

1. Cut and mount $3-5$ micron formalin-fixed paraffin-embedded tissues on positively charged slides such as Bio SB Hydrophilic Plus Slides (BSB 7028).
2. Air dry for 2 hours at $58^{\circ} \mathrm{C}$.
3. Deparaffinize, dehydrate and rehydrate tissues.
4. Subject tissues to heat induced epitope retrieval (HIER) using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030BSB 0033).
5. Any of three heating methods may be used:

## a. Tinto Retriever Pressure Cooker or Equivalent

Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA, and place on trivet in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high. Incubate for 15 minutes. Open and immediately transfer slides to room temperature.

## b. Tinto Retriever PT Module or Water Bath Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA at $95^{\circ}-99^{\circ}$. Incubate for $30-60$ minutes.

## c. Conventional Steamer Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a steamer, cover and steam for $30-60$ minutes.
6. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
7. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
8. Wash slides with ImmunoDNA washer or DI water.
9. Continue IHC staining protocol. Wash slides between each step with ImmunoDNA washer solution.

Abbreviated Immunohistochemical Protocol

| Step | ImmunoDetector <br> AP/HRP | PolyDetector <br> AP/HRP | PolyDetector <br> Plus HRP |
| :--- | :--- | :--- | :--- |
| Peroxidase/AP Blocker | 5 min. | 5 min. | 5 min |
| Primary Antibody | $30-60 \mathrm{~min}$. | $30-60 \mathrm{~min}$. | $30-60 \mathrm{~min}$. |
| 1st Step Detection | 10 min. | $30-45 \mathrm{~min}$. | 15 min. |
| 2nd Step Detection | 10 min. | Not Applicable | 15 min. |
| Substrate-Chromogen | $5-10 \mathrm{~min}$. | $5-10 \mathrm{~min}$. | $5-10 \mathrm{~min}$. |
| Counterstain/ Coverslip | Varies | Varies | Varies |

## Performance Characteristics

| Normal Tissues |  |
| :---: | :---: |
| Tositive (+) |  |
| Placenta | $10 / 10(100 \%)$ |
| Negative(-) |  |
| Myometrium | $10 / 10(100 \%)$ |
| Stomach | $0 / 7(100 \%)$ |
| Abnormal Tissues | $0 / 5(0 \%)$ |
| Hodgkin's Lymphoma |  |
| Lymphoblastic Lymphoma |  |
| Cervical Cancer |  |

## Mounting Protocols

For detailed instructions using biodegradable permanent mounting media such as XyGreen PermaMounter (BSB 0169-0174) or organic solvent based resin such as PermaMounter (BSB 0094-0097), refer to PI0174 or Pl0097.

## Product Limitations

Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized and incubation time), optimal performance should be established through the use of positive and negative controls. Results should be interpreted by a qualified medical professional.

Symbol Key / Légende des symboles/Erläuterung der Symbole

| EC REP | EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands | ${ }^{100}$ | Storage Temperature Limites de température Zulässiger Temperaturbereich |  | Manufacturer <br> Fabricant Hersteller | REF | Catalog Number Référence du catalogue Bestellnummer |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| IVD | In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum | $\square \mathbf{i}$ | Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten | 5 | Expiration Date Utiliser jusque Verwendbar bis | LOT | Lot Number Code du lot Chargenbezeichnung |

