PD-L1 Antibody
Cat. No.: 4059

Figure 1 Western Blot Validation of PD-L1 in HeLa Cells
Loading: 15 μg of lysates per lane. Antibodies: 4059 (1 μg/mL), 1 h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.

Figure 2 Independent Antibody Validation (IAV) via Protein Expression Profile in Human and Mouse cell lines
Loading: 15 μg of lysates per lane. Antibodies: 4059 (2 μg/mL), RF16035 (2 μg/mL), and beta-actin (1 μg/mL), 1 h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit and or anti-mouse IgG HRP conjugate at 1:10000 and 1:5000 dilution, respectively.

Figure 3 Validation with PD-L1 siRNA Knockdown in HeLa Cells
HeLa cells were transfected with control siRNAs (lane 1) or PD-L1 siRNAs (lane 2) Loading: 10 μg of HeLa whole cell lysates per lane. Antibodies: RF16035 (2 μg/mL) and GAPDH (3783, 0.02 μg/mL), 1 h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-mouse IgG HRP conjugate at 1:5000 dilution.

Figure 4 Validation with PD-L1 overexpression in 293 cells
Loading: Lysates/proteins at 15 μg per lane. Lane 1: non-transfected 293 cells Lane 2: PD-L1 overexpressed 293 cells Antibodies: 4059 (1 μg/mL), 1 h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.

Figure 5 Immunohistochemistry Validation of PD-L1 in Human Tonsil Cells
Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-PD-L1 antibody (4059) at 5 μg/ml. Tissue was fixed with formaldehyde and blocked with 10% serum for 1 h at RT; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody overnight at 4°C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.
Figure 6 Immunofluorescence Validation of PD-L1 in Human Heart
Immunofluorescent analysis of 4% paraformaldehyde-fixed human heart tissue labeling PD-L1 with 4059 at 20 μg/mL, followed by goat anti-rabbit IgG secondary antibody at 1/500 dilution (red). Image showing both membrane and cytoplasmic staining on human heart tissue.

Figure 7 Flow Cytometry Validation of PD-L1
Overlay histogram showing A-20 cells stained with 4059 (red line, 1μg/1x10^6 cells). 1 h incubation at 4˚C in 2% FBS/PBS. Followed by secondary antibody 488 goat anti-rabbit IgG (H+L) at 1/500 dilution for 1 h 4˚C. Isotype control antibody (Green line) was mouse IgG1 (1μg/1x10^6 cells) used under the same conditions. Acquisition of >10,000 events was performed.

Figure 8 Immunohistochemistry Validation of PD-L1 in Rat Heart
Immunohistochemical analysis of paraffin-embedded rat heart tissue using anti-PD-L1 antibody (4059) at 5 μg/ml. Tissue was fixed with formaldehyde and blocked with 10% serum for 1 h at RT; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody overnight at 4˚C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.

Figure 9 Immunohistochemistry Validation of PD-L1 in Human Heart
Immunohistochemical analysis of paraffin-embedded human heart tissue using anti-PD-L1 antibody (4059) at 2.5 μg/ml. Tissue was fixed with formaldehyde and blocked with 10% serum for 1 h at RT; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody overnight at 4˚C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.

Figure 10 Immunofluorescence Validation of PD-L1 in Rat Heart
Immunofluorescence analysis of 4% paraformaldehyde-fixed rat heart tissue labeling PD-L1 with 4059 at 20 μg/ml, followed by goat anti-rabbit IgG secondary antibody at 1/250 dilution (red).

Figure 11 Immunofluorescence Validation of PD-L1 in tumors in Human Cells (Dhar et al., 2018)
(A) Several antibody brands were first tested with RBCs, WBCs, and HeLa cells: BioLegend, ProSci, and eBioscience. ProSci (4059) was chosen as it provided the highest staining intensity.
(B, C) Using the optimal conditions of anti-PDL1 (ProSci Inc) at a concentration of 50μg/mL, following by goat anti-rabbit Alexa Fluor 647, PDL-1 staining was tested on several lung cancer cell lines: A549 (adenocarcinoma), H1703 (adenocarcinoma), H3255 (squamous) and WBCs as a control. (D) Once validated, patient samples were stained for PD-L1, CK, CD45, DAPI.
**Figure 12 Immunohistochemistry Validation of PD-L1 in Human Tumors (Gadiot et al., 2011)**

Immunohistochemical analysis of patient tumors labeling PD-L1 with anti-PD-L1 antibodies (4059). Several anti-PD-L1 antibodies were tested for staining. “Only 1 antibody gave no background staining and was competitively blocked by the addition of PD-L1Fc protein (ProSci, #4059).”

### Specifications

<table>
<thead>
<tr>
<th><strong>HOST SPECIES:</strong></th>
<th>Rabbit</th>
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<tbody>
<tr>
<td><strong>SPECIES REACTIVITY:</strong></td>
<td>Human, Mouse, Rat</td>
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<tr>
<td><strong>HOMOLOGY:</strong></td>
<td>Predicted species reactivity based on immunogen sequence: Rat: (77%), Mouse: (71%)</td>
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<td><strong>IMMUNOGEN:</strong></td>
<td>Anti-PD-L1 antibody (4059) was raised against a peptide corresponding to 17 amino acids near the center of human PD-L1 isoform 1. The immunogen is located within amino acids 60 - 110 of PD-L1.</td>
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<td><strong>TESTED APPLICATIONS:</strong></td>
<td>ELISA, Flow, IF, IHC-P, WB</td>
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<td><strong>APPLICATIONS:</strong></td>
<td>WB: 1-2 μg/mL; IHC-P: 2.5-5 μg/mL; IF: 20 μg/mL; Flow Cyt: 0.5 μg/mL. Antibody validated: Western Blot in human and mouse samples; Immunohistochemistry in human and rat samples; Immunofluorescence in human and rat samples and Flow Cytometry in mouse samples. All other applications and species not yet tested.</td>
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<tr>
<td><strong>SPECIFICITY:</strong></td>
<td>PD-L1 antibody has no cross-reactivity to PD-L2.</td>
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| **POSITIVE CONTROL:** | 1) Cat. No. 1201 - HeLa Cell Lysate  
2) Cat. No. 1224 - Daudi Cell Lysate  
3) Cat. No. 1204 - K562 Cell Lysate  
4) Cat. No. 10-501 - Human Heart Tissue Slide  
5) Cat. No. 1301 - Human Heart Tissue Lysate |
| **PREDICTED MOLECULAR WEIGHT:** | Predicted: 33 kDa  
Observed: 37 kDa |
**VALIDATION:**

Independent Antibody Validation (Figure 2) shows similar PD-L1 expression profile in both human and mouse cell lines detected by two independent anti-PD-L1 antibodies that recognize different epitopes, 4059 against the center of human PD-L1 and RF16035 against the extracellular domain. PD-L1 proteins are detected in most of the cell lines, but not in A549 and THP-1 cells by the two independent antibodies.

siRNA Knockdown Validation (Figure 3): Anti-PD-L1 antibody (4059) specificity was further verified by PD-L1 specific siRNA knockdown. PD-L1 signal in HeLa cells transfected with PD-L1 siRNAs was much weaker in comparison with that in HeLa cells transfected with control siRNAs.

Overexpression Validation (Figure 4): Anti-PD-L1 antibodies (4059) can detect the overexpression of PD-L1 protein in 293 cells transfected with PD-L1.

**ISOFORMS:**

Human PD-L1 has 3 isoforms, including isoform 1 (290aa, 33.3kD), isoform 2 (176aa, 20.2kD), and isoform 3 (178aa, 20.5kD). This antibody detects human isoform 1&3, mouse and rat PD-L1 (290aa, 33kD for both of them).

**Properties**

- **PURIFICATION:** PD-L1 Antibody is affinity chromatography purified via peptide column.
- **CLONALITY:** Polyclonal
- **ISOTYPE:** IgG
- **CONJUGATE:** Unconjugated
- **PHYSICAL STATE:** Liquid
- **BUFFER:** PD-L1 Antibody is supplied in PBS containing 0.02% sodium azide.
- **CONCENTRATION:** 1 mg/mL
- **STORAGE CONDITIONS:** PD-L1 antibody can be stored at 4˚C for three months and -20˚C, stable for up to one year. As with all antibodies care should be taken to avoid repeated freeze thaw cycles. Antibodies should not be exposed to prolonged high temperatures.

**Additional Info**

- **OFFICIAL SYMBOL:** CD274
- **ALTERNATE NAMES:** PD-L1 Antibody: B7-H, B7H1, PDL1, PD-L1, PDCD1L1, PDCD1LG1, Programmed cell death 1 ligand 1, B7 homolog 1
- **ACCESSION NO.:** NP_054862
- **PROTEIN GI NO.:** 7661534
- **GENE ID:** 29126
- **USER NOTE:** Optimal dilutions for each application to be determined by the researcher.

**Background and References**

BACKGROUND:
PD-L1 plays a critical role in induction and maintenance of immune tolerance to self. As a ligand for the inhibitory receptor PDCD1/CD279, PD-L1 modulates the activation threshold of T-cells and limits T-cell effector response (1). The PDCD1/CD279-mediated inhibitory pathway is exploited by tumors to attenuate anti-tumor immunity and facilitate tumor survival (2,3). Through a yet unknown activating receptor, it may costimulate T-cell subsets that predominantly produce interleukin-10 (IL10) (4).

REFERENCES:

CITATIONS:
3) Angell et al. BRAF V600E in papillary thyroid carcinoma is associated with increased programmed death ligand 1 expression and suppressive immune cell infiltration. Thyroid. 2014; 24(9):1385-93PMID: 24955518
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