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 &#956g/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

**HOST SPECIES:** Rabbit

**SPECIES REACTIVITY:** Human, Mouse, Rat

**HOMOLOGY:** Predicted species reactivity based on immunogen sequence: Rat: (77%), Mouse: (71%)

**IMMUNOGEN:** 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.

**TESTED APPLICATIONS:** ELISA, Flow, IF, IHC-P, WB

**APPLICATIONS:** 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.

**SPECIFICITY:** PD-L1 antibody has no cross-reactivity to PD-L2.

**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

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<td><strong>ISOTYPE:</strong></td>
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<td><strong>CONJUGATE:</strong></td>
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<td><strong>PHYSICAL STATE:</strong></td>
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<td><strong>BUFFER:</strong></td>
<td>PD-L1 Antibody is supplied in PBS containing 0.02% sodium azide.</td>
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<td><strong>CONCENTRATION:</strong></td>
<td>1 mg/mL</td>
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<td><strong>STORAGE CONDITIONS:</strong></td>
<td>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.</td>
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### Additional Info

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<td><strong>USER NOTE:</strong></td>
<td>Optimal dilutions for each application to be determined by the researcher.</td>
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### 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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