

Anti-PD-L1 Antibody [PSH04-80]

HA722184



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Mouse
Applications:	WB, IHC-P, mIHC
Molecular Wt:	Predicted band size: 33 kDa
Clone number:	PSH04-80

Description: Programmed death-ligand 1 (PD-L1) also known as cluster of differentiation 274 (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 40kDa type 1 transmembrane protein that has been speculated to play a major role in suppressing the adaptive arm of immune systems during particular events such as pregnancy, tissue allografts, autoimmune disease and other disease states such as hepatitis. Normally the adaptive immune system reacts to antigens that are associated with immune system activation by exogenous or endogenous danger signals. In turn, clonal expansion of antigen-specific CD8+ T cells and/or CD4+ helper cells is propagated. The binding of PD-L1 to the inhibitory checkpoint molecule PD-1 transmits an inhibitory signal based on interaction with phosphatases (SHP-1 or SHP-2) via Immunoreceptor Tyrosine-Based Switch Motif (ITSM). This reduces the proliferation of antigen-specific T-cells in lymph nodes, while simultaneously reducing apoptosis in regulatory T cells (anti-inflammatory, suppressive T cells) – further mediated by a lower regulation of the gene Bcl-2.

Immunogen: Recombinant protein within mouse PD-L1 aa 1-250 / 290.

Positive control: RAW264.7 treated with 10µg/mL LPS for 8 hours cell lysate, J774A.1 treated with 1µg/mL LPS for 24 hours cell lysate, mouse spleen tissue lysate, mouse thymus tissue, mouse lung tissue.

Subcellular location: Cell membrane, Early endosome membrane, Recycling endosome membrane.

Database links: SwissProt: Q9EP73 Mouse

Recommended Dilutions:

WB	1:2,000
IHC-P	1:200
mIHC	1:500

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4°C after thawing. Aliquot store at -20°C. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

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Images

Fig1: Western blot analysis of PD-L1 on different lysates with Rabbit anti-PD-L1 antibody (HA722184) at 1/2,000 dilution.

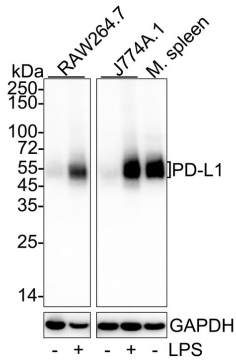
Lane 1: RAW264.7 cell lysate

Lane 2: RAW264.7 treated with 10µg/mL LPS for 8 hours cell lysate

Lane 3: J774A.1 cell lysate

Lane 4: J774A.1 treated with 1µg/mL LPS for 24 hours cell lysate

Lane 5: Mouse spleen tissue lysate



Lysates/proteins at 30 µg/Lane.

Predicted band size: 33 kDa

Observed band size: 45-60 kDa

Exposure time: 46 seconds; ECL: K1802;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722184) at 1/2,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

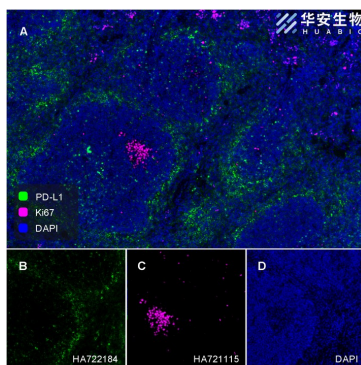


Fig2: Fluorescence multiplex immunohistochemical analysis of mouse spleen (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-PD-L1 (HA722184, Green) and anti-Ki67 (HA721115, Violet) on spleen. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in two rounds of staining: in the order of HA722184 (1/500 dilution) and HA721115 (1/2,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

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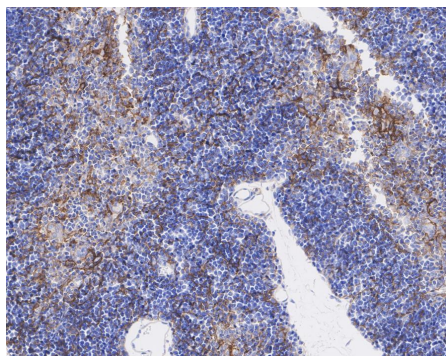


Fig3: Immunohistochemical analysis of paraffin-embedded mouse thymus tissue with Rabbit anti-PD-L1 antibody (HA722184) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA722184) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

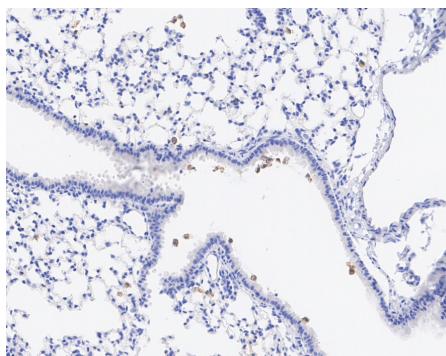


Fig4: Immunohistochemical analysis of paraffin-embedded mouse lung tissue with Rabbit anti-PD-L1 antibody (HA722184) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA722184) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Lei Q et al. Resistance Mechanisms of Anti-PD1/PDL1 Therapy in Solid Tumors. *Front Cell Dev Biol.* 2020 Jul
2. Tran-Nguyen VK et al. Structure-based virtual screening for PDL1 dimerizers: Evaluating generic scoring functions. *Curr Res Struct Biol.* 2022 Jun

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