PD-L1

Western Blot

https://www.abcam.com/protocols/general-western-blot-protocol

Target Overview in WB Application

PD-L1 is heavily glycosylated, therefore the **actual band size in WB is between 40-60kD**, different from the predicted 20-33kD.

Here are a few tips to help ensure the best results in WB:

Sample Preparation	•Add adequate **protease inhibitors** (or phosphatase inhibitors for proteins modified by phosphorylation) to avoid target protein degradation.
	•**Ultrasonicate** samples to enrich more target proteins.
	•Keep samples on **ice** during the whole WB process.
	•Perform a Bradford assay, a Lowry assay or a bicinchoninic acid (BCA) assay to **determine the protein concentration**.
Electrophoresis	•Load **20-50µg** total protein per lane.
Transferring	•Use **Ponceau S staining** to determine if the transfer is successful.

You should pay attention to these notes to maximize the signal:

- ✓ **Treat samples with PNGase F** to confirm the specificity of bands if necessary.

  PMID: 31327656

- ✓ Actual band size in Western Blot is between **40-60kD**.

Immunohistochemistry

https://www.abcam.com/protocols/immunostaining-paraffin-frozen-free-floating-protocol

Target Overview in IHC Application

PD-L1 is highly expressed **in lung, ovary and colon carcinoma and in melanomas**. However, not all samples show positive signal. And the glycosylation of PD-L1 may interfere antibody binding.

Here are a few tips to help ensure the best results in IHC:

Sample Fixation	●The ideal fixation time will depend on the size of the tissue block and the type of tissue, but **fixation between 18–24h** is suitable for most samples.
	●Under-fixation can lead to edge staining, with strong signal on the edges of the section and no signal in the middle.
	●Over-fixation can mask the epitope; antigen retrieval can help reverse this masking, but if the tissue has been fixed for a long period of time (i.e. over a weekend), there may be no signal even after antigen retrieval.
Antibody Incubation	●It is recommended to optimize antibody dilution in preliminary experiments according to datasheets.

You should pay attention to these notes to maximize the signal:

- ✓ Treat samples with **PNGase F** may increase signal intensity. (PMID: 31327656)
- ✓ To find PD-L1 expression in lung cancer tissues, please refer to the table below (PD-L1 IHC 28-8 pharmDx, Dako). For PD-L1 expression in other tumors, please refer to https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3991103/

Tumors	Positive Samples/Total Samples	Positive Ratio
Lung Adenocarcinoma	2/5	40%
Lung Squamous-Cell Carcinoma	1/3	33%
Small Cell Lung Carcinoma	1/2	50%

Protein Function	Plays a critical role in induction and maintenance of immune tolerance to self (PMID:11015443, 28813417, 28813410). As a ligand for the inhibitory receptor PDCD1/PD-1, modulates the activation threshold of T-cells and limits T-cell effector response (PMID:11015443, 28813417, 28813410). Through a yet unknown activating receptor, may co-stimulate T-cell subsets that predominantly produce interleukin-10 (IL10) (PMID:10581077). The PDCD1-mediated inhibitory pathway is exploited by tumors to attenuate anti-tumor immunity and escape destruction by the immune system, thereby facilitating tumor survival (PMID:28813417, 28813410). SwissProt: Q9NZQ7
Expression	Highly expressed in the **heart, skeletal muscle, placenta and lung.** Weakly expressed in the thymus, spleen, kidney and liver. Expressed on activated T- and B-cells, dendritic cells, keratinocytes and monocytes. PMID: 10581077, 11015443 Up-regulated on T- and B-cells, dendritic cells, keratinocytes and monocytes after LPS and IFNG activation. Up-regulated in B-cells activated by surface Ig cross-linking. PMID: 10581077, 11015443, 28813410
Location	Cell membrane (PMID: 28813417, 28813410), early endosome membrane, recycling endosome membrane, associates with CMTM6 at recycling endosomes, where it is protected from being targeted for lysosomal degradation (PMID: 28813417). Isoform 1: Cell membrane (PMID: 15780196) Isoform 2: Endomembrane system (PMID: 15780196)
Isoforms	Human Isoform I-3: 20-33kD (predicted) Mouse: 32.8kD (predicted) **The observed band size of PD-L1 may not be the same as predicted MWs in WB due to the different forms of PD-L1.**
Modifications	Glycosylation (PMID: 19159218) Heterodimers (PMID: 18287011, 26602187) Ubiquitination; STUB1 likely mediates polyubiquitination of PD-L1/CD274 triggering its degradation (PMID: 28813410). **The observed band size of PD-L1 may not be the same as predicted MWs in WB due to these modifications.**
Positive Controls	**WB:** B-CPAP, MD-MB-231, U87-MG **IHC:** Human tonsil, head and neck squamous cell carcinoma, placenta tissues
Negative Controls	**WB:** A549 SW480 **IHC:** MCF-7 (PMID: 31327656)