CD31

Western Blot

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

Target Overview in WB Application

CD31 is a **transmembrane protein** (PMID:21464369, 17580308) which requires special treatment of samples in Western Blot. It contains many post-transcriptional modifications, including glycosylations and phosphorylations (PMID:26702061, 19349973, 19159218, 19342684, 21464369, 9298995), making the **actual band size is around 130kD**, different from the predicted 79-83kD.

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.
	•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	•For large proteins (the MW of target protein >100 kDa), be sure to run samples in 8% or lower separating gel.
	•Load **20-50µg** total protein per lane.
Transferring	•It is preferred to **add SDS** to a final concentration of 0.1% in the transfer buffer for large proteins.
	•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 or phosphatase** to confirm the specificity of bands if necessary.
- ✓ Actual band size is **around 130kD**.

Immunohistochemistry

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

Target Overview in IHC Application

CD31 is widely expressed on **platelets and leukocytes** and is primarily concentrated at the borders between endothelial cells (PMID:18388311, 21464369). It can be used to help identify blood vessels and endothelial cells. Pathologists use it to identify vascular origin of tumors, but nodal sinuses may show signal as well (PMID:12890824). CD31 is also used to identify vascular invasion (PMID:14514787, 15737030) and assess the micro-vessel density of tumors (PMID:18343785).

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.
Antibody Incubation	●It is recommended to **optimize antibody dilution** in preliminary experiments according to datasheets.

Immunocytochemistry/Immunofluorenscence

https://www.abcam.com/protocols/immunocytochemistry-immunofluorescence-protocol

Target Overview in ICC/IF Application

CD31 expresses in endothelial cells, B cells, platelets, macrophages, monocytes, NK cells, T cells (PMID:18388311, 21464369). It is one of the biomarkers of endothelial cells. It is widely used to confirm the cell type and co-localization of proteins of interest in immunocytochemistry application.

Here are a few tips to help ensure the best results in ICC/IF:

Sample Fixation	● It is recommended to fix cells in 4% PFA for 20 minutes at room temperature.
	●Please **do not over-fix** your samples, which will reduce signal.
Permeabilization	● It is recommended to incubate cells with **0.1% Triton-X for 5 min** to detect nuclear antigen.
Antibody Incubation	● Use **0.3M glycine** to quench autofluorescence caused by aldehydes.

Protein Function	Cell adhesion molecule which is required for leukocyte transendothelial migration (TEM) under most inflammatory conditions (PMID:19342684, 17580308). Trans-homophilic interaction may play a role in endothelial cell-cell adhesion via cell junctions (PMID:27958302). Heterophilic interaction with CD177 plays a role in transendothelial migration of neutrophils (PMID:17580308). Homophilic ligation of PECAM1 prevents macrophage-mediated phagocytosis of neighboring viable leukocytes by transmitting a detachment signal (PMID:12110892). Promotes macrophage-mediated phagocytosis of apoptotic leukocytes by tethering them to the phagocytic cells; PECAM1-mediated detachment signal appears to be disabled in apoptotic leukocytes (PMID:12110892). Modulates bradykinin receptor BDKRB2 activation (PMID:18672896). Regulates bradykinin- and hyperosmotic shock-induced ERK1/2 activation in endothelial cells (PMID:18672896). SwissProt: P16284
Expression	Expressed on platelets, leukocytes. Primarily concentrated at the borders between endothelial cells (PMID:18388311, 21464369). Expressed on neutrophils (PMID:17580308), human umbilical vein endothelial cells (HUVECs) (PMID:19342684, 17580308). Isoform Long predominates in all tissues examined (PMID:12433657).
Location	Cell membrane (PMID: 17580308) Cell surface expression on neutrophils is down-regulated upon fMLP or CXCL8/IL8-mediated stimulation (PMID: 17580308).
Isoforms	Human: Isoform 1-6: 79-83kD (predicted) Mouse: Isoform 1-4: 69.8-81.3kD (predicted) **The observed band size of CD31 may not be the same as predicted MWs in WB due to the different forms of CD31.**
Modifications	Glycosylation (PMID:26702061, 19349973, 19159218) Phosphorylation (PMID:21464369, 9298995, 19342684, 18710921) Palmitoylation (PMID: 17139370, 22496122) **The observed band size of CD31 may not be the same as predicted MWs in WB due to these modifications.**
Positive Controls	**WB:** HUVEC and Jurkat cell lysates (ab7899). Human spleen and kidney tissue lysate. **IHC:** Human tonsil tissue. **ICC:** HUVEC cells.
Negative Controls	**WB:** NIH/3T3 whole cell lysate (ab7179)