

NLRP3

Western Blot

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

Target Overview in WB Application

NLRP3 is regulated by post-translational modifications, such as ubiquitination and phosphorylation. It has several isoforms in human and mouse. Therefore, it's **may exist several bands** in WB assay. Moreover, the predicted molecular weight of NLRP3 is about 118 kDa. The **8% gel** is more appropriate. Large proteins (>100 kDa) will tend to precipitate in the gel, hindering transfer. **Adding SDS** to a final concentration of 0.1% **in the transfer buffer** will facilitate transfer. Methanol tends to remove SDS from proteins, so **reducing the methanol** percentage to 10% or less will also prevent precipitation.

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

Sample Preparation	<ul style="list-style-type: none"> • Add adequate protease inhibitors (or phosphatase inhibitors for proteins modified by phosphorylation) to avoid target protein degradation.
	<ul style="list-style-type: none"> • Ultrasonicate samples to enrich more target proteins.
	<ul style="list-style-type: none"> • Keep samples on ice during the whole WB process.
	<ul style="list-style-type: none"> • Perform a Bradford assay, a Lowry assay or a bicinchoninic acid (BCA) assay to determine the protein concentration.
Electrophoresis	<ul style="list-style-type: none"> • For large proteins (the MW of target protein >100 kDa), be sure to run samples in 8% or lower separating gel.
Transferring	<ul style="list-style-type: none"> • It is preferred to add SDS to a final concentration of 0.1% in the transfer buffer for large proteins.
	<ul style="list-style-type: none"> • Wash PVDF membrane to remove methanol completely.
	<ul style="list-style-type: none"> • To determine if the transfer is successful by visualization of proteins in membranes using Ponceau S.

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

- ✓ The level of NLRP3 expression can be elevated when cells are **treated with LPS**, as described in the literature. (PMID: 22569257)
- ✓ There **may be multiple bands** of NLRP3 in WB assay. NLRP3 can be regulated at post-translational level, such as phosphorylation, ubiquitination. And NLRP3 has 6 isoforms in human(predicted).
- ✓ Expressed in blood leukocytes. **Strongly expressed in polymorphonuclear cells and osteoblasts**. Undetectable or expressed at a lower magnitude in B- and T-lymphoblasts, respectively. **High level of expression detected in chondrocytes**. Detected in non-keratinizing epithelia of oropharynx, esophagus and ectocervix and in the urothelial layer of the bladder. (SwissProt: Q96P20)

Protein Function	<p>May function as an inducer of apoptosis. Interacts selectively with ASC and this complex may function as an upstream activator of NF-kappa-B signaling. Inhibits TNF-alpha induced activation and nuclear translocation of RELA/NF-KB p65. Also inhibits transcriptional activity of RELA. Activates caspase-1 in response to a lot of triggers including bacterial or viral infection which leads to processing and release of IL1B and IL18.</p> <p style="text-align: right;">SwissProt: Q96P20</p>
Expression	<p>Expressed in blood leukocytes. Strongly expressed in polymorphonuclear cells and osteoblasts. Undetectable or expressed at a lower magnitude in B- and T-lymphoblasts, respectively. High level of expression detected in chondrocytes. Detected in non-keratinizing epithelia of oropharynx, esophagus and ectocervix and in the urothelial layer of the bladder.</p>
Location	<p>Cytoplasm</p>
Isoforms	<p>Human Isoform 1: 84 kD (predicted) Isoform 2: 106 kD (predicted) Isoform 3-6: 110-120 kD (predicted)</p> <p>Mouse Isoform 1: 96 kD (predicted) Isoform 2-4: 110-120 kD (predicted)</p> <p>Rat Isoform 1: 119 kD (predicted)</p> <p>The observed band size of NLRP3 may not be the same as predicted MWs in WB due to the different forms of NLRP3.</p>
Modifications	<p>Phosphorylation/Ubiquitination</p> <p>The observed band size of NLRP3 may not be the same as predicted MWs in WB due to these modifications.</p>
Positive Controls	<p>WB Human THP1 WT cells treated with 200 ng/ml LPS for 3h RAW 264.7 (+/- treatment with LPS) ab7187 Rat spleen lysate</p>
Negative Controls	<p>Jurkat (human t cell leukemia t lymphocyte) whole cell lysate</p>