

DESCRIPTION

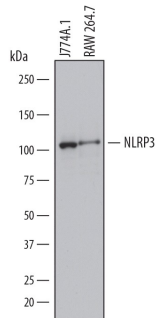
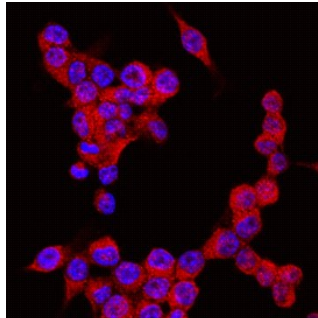
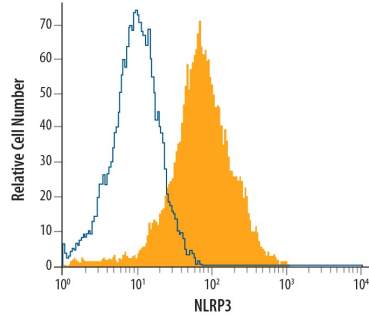
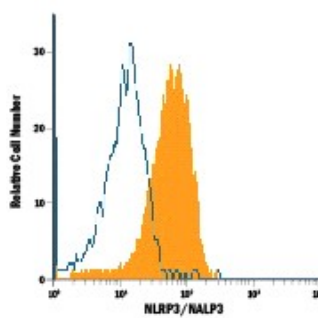
Species Reactivity	Human/Mouse
Specificity	Detects mouse NLRP3/NALP3 in direct ELISAs. In direct ELISAs, 100% cross-reactivity with recombinant human NLRP3/NALP3 is observed.
Source	Monoclonal Rat IgG _{2A} Clone # 768319
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>E. coli</i> -derived recombinant mouse NLRP3/NALP3 Met1-Arg153 Accession # Q8R4B8
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	2 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10 ⁶ cells	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

DATA

<p>Western Blot</p>  <p>Detection of Mouse NLRP3/NALP3 by Western Blot. Western blot shows lysates of J774A.1 mouse reticulum cell sarcoma macrophage cell line and RAW 264.7 mouse monocyte/macrophage cell line. PVDF membrane was probed with 2 µg/mL of Rat Anti-Human/Mouse NLRP3/NALP3 Monoclonal Antibody (Catalog # MAB7578) followed by HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # HAF005). A specific band was detected for NLRP3/NALP3 at approximately 117 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunocytochemistry</p>  <p>NLRP3/NALP3 in RAW 264.7 Mouse Cell Line. NLRP3/NALP3 was detected in immersion fixed RAW 264.7 mouse monocyte/macrophage cell line using Rat Anti-Human/Mouse NLRP3/NALP3 Monoclonal Antibody (Catalog # MAB7578) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the Northern-Lights™ 557-conjugated Anti-Rat IgG Secondary Antibody (red; Catalog # NL013) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.</p>
<p>Intracellular Staining by Flow Cytometry</p>  <p>Detection of NLRP3/NALP3 in RAW 264.7 Mouse Cell Line by Flow Cytometry. RAW 264.7 mouse monocyte/macrophage cell line was stained with Rat Anti-Human/Mouse NLRP3/ NALP3 Monoclonal Antibody (Catalog # MAB7578, filled histogram) or isotype control antibody (Catalog # MAB006, open histogram), followed by Allophycocyanin-conjugated Anti-Rat IgG Secondary Antibody (Catalog # F0113). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with saponin.</p>	<p>Intracellular Staining by Flow Cytometry</p>  <p>Detection of NLRP3/NALP3 in Human Monocytes by Flow Cytometry. Human peripheral blood monocytes was stained with Rat Anti-Human/Mouse NLRP3/NALP3 Monoclonal Antibody (Catalog # MAB7578, filled histogram) or isotype control antibody (Catalog # MAB006, open histogram), followed by Allophycocyanin-conjugated Anti-Rat IgG Secondary Antibody (Catalog # F0113). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with saponin.</p>

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.5 mg/mL.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

NLRP3, also known as NALP3, CIAS1, PYPAF or Cryopyrin, is a cytosolic ~120 kDa member of the NLRP family of proteins expressed in leukocytes, especially neutrophils. As a component of the inflammasome, NLRP3 activates caspases 1 and 5. Defects in NLRP3 may cause FCAS1, CINCA, or Muckle-Wells autoinflammatory syndromes. Mouse NLRP3 contains an N-terminal Pyrin domain (aa 1-91) followed by a Nacht region (aa 216-532), and seven LRRs (aa 739-988). Within the sequence used as an immunogen, mouse NLRP3 shares 78% and 93% aa identity with human and rat NLRP3, respectively. Alternate splicing of mouse NLRP3 generates additional isoforms that lack either LRR2 and 3, LRR 6 and 7, or LRR4-9.