abcam

Product datasheet

Anti-NLRP3 antibody [EPR23094-1] ab263899



★★★★ <u>2 Abreviews</u> <u>146 References</u> 画像数 12

製品の概要

製品名 Anti-NLRP3 antibody [EPR23094-1]

製品の詳細 Rabbit monoclonal [EPR23094-1] to NLRP3

由来種 Rabbit

特異性 NLRP3 is related with inflammation and is cell-type dependent. This antibody detects no signal or

very weak signal in most non-diseased tissue samples and untreated cell lysates. Stimulation may

be required to allow detection of the NLRP3 protein in most samples, as described in the

literature (PMID: 29070054, 29434227, and 22569257).

FURTHER INFORMATION ON SPECIFIC NOTE (Chinese Version)

アプリケーション 適用あり: WB, IP, Flow Cyt (Intra), Indirect ELISA

適用なし: ICC/IF or IHC-P

種交差性 交差種: Mouse, Rat, Human

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール WB: THP-1 and J774A.1 whole cell lysate. LPS treated RAW 264.7 whole cell lysate. Rat

> spleen lysate. Mouse Wt BMDCs treated with 50 ng/ml LPS, 3h; Human THP1 Wt cells treated with 200 ng/ml LPS, 3h. RAW 264.7 (Mouse Abelson murine leukemia virusinduced tumor macrophage) whole cell lysate. IP: RAW 264.7 and THP-1 whole cell

lysate. Flow Cyt (intra): THP-1 cells.

特記事項 This product is a recombinant monoclonal antibody, which offers several advantages

including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

バッファー pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

精製度 Protein A purified

ポリ/モノ モノクローナル **ウローン名** EPR23094-1

アイソタイプ lgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab263899の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB	★★★★☆ (2)	1/1000. Predicted molecular weight: 118 kDa. Stimulation may be required to allow detection of the NLRP3 protein in most samples.
		There may be multiple bands of NLRP3 in WB assay due to different isoforms and PTMs.
IP		1/30.
Flow Cyt (Intra)		1/50.
Indirect ELISA		Use at an assay dependent concentration.

追加情報 Is unsuitable for ICC/IF or IHC-P.

ターゲット情報

機能 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 number of triggers including bacterial or viral infection which

leads to processing and release of L1B and L18.

組織特異性 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.

関連疾患 Defects in NLRP3 are the cause of familial cold autoinflammatory syndrome type 1 (FCAS1)

[MIM:120100]; also known as familial cold urticaria. FCAS are rare autosomal dominant systemic inflammatory diseases characterized by episodes of rash, arthralgia, fever and conjunctivitis after

generalized exposure to cold.

Defects in NLRP3 are a cause of Muckle-Wells syndrome (MWS) [MIM:191900]; also known as

urticaria-deafness-amyloidosis syndrome. MWS is a hereditary periodic fever syndrome

characterized by fever, chronic recurrent urticaria, arthralgias, progressive sensorineural deafness, and reactive renal amyloidosis. The disease may be severe if generalized amyloidosis occurs.

Defects in NLRP3 are the cause of chronic infantile neurologic cutaneous and articular syndrome (CINCA) [MIM:607115]; also known as neonatal onset multisystem inflammatory disease (NOMID). CINCA is a rare congenital inflammatory disorder characterized by a triad of neonatal onset of cutaneous symptoms, chronic meningitis and joint manifestations with recurrent fever and inflammation.

配列類似性 Belongs to the NLRP family.

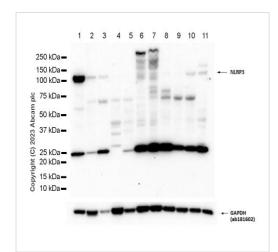
Contains 1 DAPIN domain.

Contains 9 LRR (leucine-rich) repeats.

Contains 1 NACHT domain.

細胞内局在 Cytoplasm.

画像



Western blot - Anti-NLRP3 antibody [EPR23094-1] (ab263899)

All lanes : Anti-NLRP3 antibody [EPR23094-1] (ab263899) at 1/1000 dilution

Lane 1: Rat spleen tissue lysate

Lane 2: Rat thymus tissue lysate

Lane 3: Rat lung tissue lysate

Lane 4: Rat heart tissue lysate

Lane 5: Rat kidney tissue lysate

Lane 6: Rat brain tissue lysate

Lane 7: Rat hippocampus tissue lysate

Lane 8: Rat spinal cord tissue lysate

Lane 9: Rat placenta tissue lysate

Lane 10: Rat ovary tissue lysate

Lane 11: Rat liver tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000

dilution

Predicted band size: 118 kDa Observed band size: 118 kDa

Exposure time: 180 seconds

Blocking and dilution buffer: 5% NFDM /TBST. NLRP3 is

expressed at a much low level in normal tissues (PMID: 33538177,

PMID: 25524927, PMID: 20688930, PMID: 35676979, PMID:

31923221, PMID: 24166187).

All lanes : Anti-NLRP3 antibody [EPR23094-1] (ab263899) at 1/1000 dilution

Lane 1: Mouse thymus tissue lysate

Lane 2: Mouse lung tissue lysate

Lane 3: Mouse heart tissue lysate

Lane 4: Mouse kidney tissue lysate

Lane 5: Mouse brain tissue lysate

Lane 6: Mouse placenta tissue lysate

Lane 7: Mouse ovary tissue lysate

Lane 8: Mouse liver tissue lysate

Lane 9: Raw 264.7 (Mouse Abelson murine leukemia virus-

induced tumor macrophage) whole cell lysate

Western blot - Anti-NLRP3 antibody [EPR23094-1] (ab263899)

250 kDa — 150 kDa — 100 kDa —

전 75 kDa 🗕

50 kDa -

25 kDa — 20 kDa — 15 kDa — 10 kDa —

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/20000

dilution

Predicted band size: 118 kDa **Observed band size:** 118 kDa

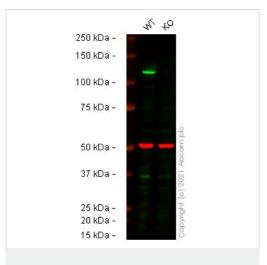
Exposure time: 180 seconds

Blocking and Diluting buffer: 5% NFDM/TBST

Lane 9 Exposure Time: 20 seconds

NLRP3 is expressed at a much low level in normal tissues (PMID: 33538177, PMID: 25524927, PMID: 20688930, PMID: 35676979,

PMID: 31923221, PMID: 24166187).



Western blot - Anti-NLRP3 antibody [EPR23094-1] (ab263899)

All lanes : Anti-NLRP3 antibody [EPR23094-1] (ab263899) at 1/500 dilution

Lane 1: Wild-type THP-1 cell lysate

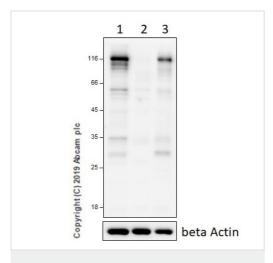
Lane 2: NLRP3 knockout THP-1 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 118 kDa **Observed band size:** 118 kDa

False colour image of Western blot: Anti-NLRP3 antibody [EPR23094-1] staining at 1/500 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab263899 was shown to bind specifically to NLRP3. A band was observed at 118 kDa in wild-type THP-1 cell lysates with no signal observed at this size in NLRP3 knockout cell line ab280063 (knockout cell lysate ab280122). To generate this image, wild-type and NLRP3 knockout THP-1 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216772) at 1/20000 dilution.



Western blot - Anti-NLRP3 antibody [EPR23094-1] (ab263899)

This image is courtesy of Benedikt Saller, from Prof. Dr. Olaf Groß' lab at the University of Freiburg.

All lanes : Anti-NLRP3 antibody [EPR23094-1] (ab263899) at 1/1000 dilution

Lane 1: Wild-type mouse BMDCs treated with 50 ng/ml LPS

Lane 2 : NLRP3 knockout mouse BMDCs treated with 50 ng/ml LPS

Lane 3: Wild-type human THP1 cells treated with 200 ng/ml LPS

Lysates/proteins at 25000 cells per lane.

Secondary

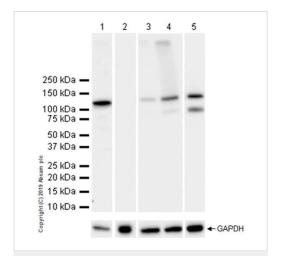
All lanes: Goat anti-Rabbit HRP at 1/5000 dilution

Developed using the ECL technique.

Predicted band size: 118 kDa

Exposure time: 10 seconds

Blocking step: 2% SMP in PBS/T for 60 minutes at RT.



Western blot - Anti-NLRP3 antibody [EPR23094-1] (ab263899)

All lanes : Anti-NLRP3 antibody [EPR23094-1] (ab263899) at 1/1000 dilution

Lane 1 : THP-1 (human monocytic leukemia monocyte) whole cell lysate at 20 µg

Lane 2 : Jurkat (human t cell leukemia t lymphocyte) whole cell lysate at 20 µg

Lane 3 : Untreated RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 10 μg

Lane 4 : RAW 264.7 treated with 10 μ g/ml LPS for 8 hours, whole cell lysate at 10 μ g

Lane 5 : J774A.1 (mouse reticulum cell sarcoma monocyte macrophage) whole cell lysate at 20 μg

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 118 kDa

The molecular weight/expression profile observed is consistent with what has been described in the literature (PMID: 26939933, 30315268).

Note: The band around 75kDa is the short form of NLRP3 (NLRP3s).

Low expression cell line: Jurkat (PMID: 19767079).

Exposure times: Lane 1: 48 secs; Lane 2: 3 min; Lanes 3-4: 1 sec; Lane 5: 6 secs.

All lanes : Anti-NLRP3 antibody [EPR23094-1] (ab263899) at 1/1000 dilution

Lane 1: C6 (Rat glial tumor glial cell) whole cell lysate

Lane 2: RAW 264.7 (Mouse Abelson murine leukemia virus-

induced tumor macrophage) whole cell lysate

Lane 3 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate

Lane 4: NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

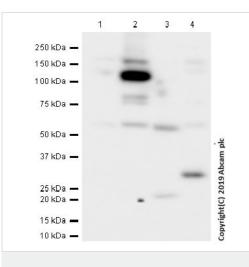
All lanes: Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/10000

dilution

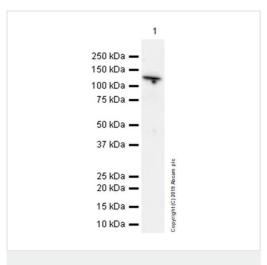
Predicted band size: 118 kDa **Observed band size:** 118 kDa

Exposure time: 15 seconds

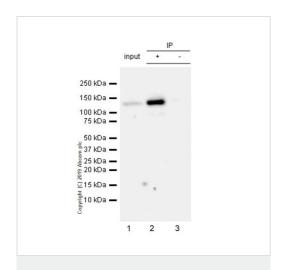
Stimulation is required to allow detection of the NLRP3 protein in C6, PC-12 and NIH/3T3 cell lines.



Western blot - Anti-NLRP3 antibody [EPR23094-1] (ab263899)



Western blot - Anti-NLRP3 antibody [EPR23094-1] (ab263899)



Immunoprecipitation - Anti-NLRP3 antibody [EPR23094-1] (ab263899)

Anti-NLRP3 antibody [EPR23094-1] (ab263899) at 1/1000 dilution + Rat spleen lysate at 10 μg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 118 kDa

Exposure time: 3 minutes

The molecular weight/expression profile observed is consistent with what has been described in the literature (PMID: 26939933, 30315268).

NLRP3 was immunoprecipitated from 0.35 mg RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate with ab263899 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab263899 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/1000 dilution.

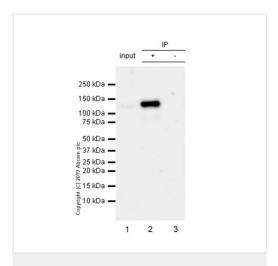
Lane 1: RAW 264.7 whole cell lysate 10µg

Lane 2: ab263899 IP in RAW 264.7 whole cell lysate

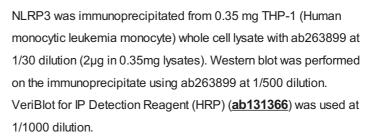
Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab263899 in RAW 264.7 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds.



Immunoprecipitation - Anti-NLRP3 antibody [EPR23094-1] (ab263899)



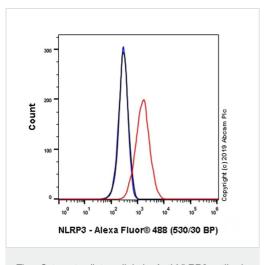
Lane 1: THP-1 whole cell lysate 10µg

Lane 2: ab263899 IP in THP-1 whole cell lysate

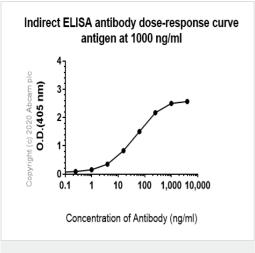
Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab263899 in THP-1 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 30 seconds.

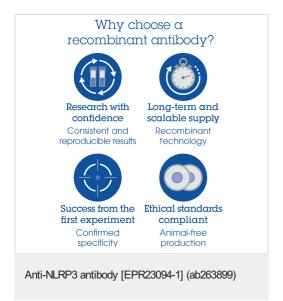


Flow Cytometry (Intracellular) - Anti-NLRP3 antibody [EPR23094-1] (ab263899) Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized THP-1 (Human monocytic leukemia monocyte) cells labeling NLRP3 with ab263899 at 1/50 (Red) compared with a Rabbit monoclonal IgG (ab172730) / Black isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor[®]488, ab150077) at 1/2000 dilution was used as the secondary antibody.



Indirect ELISA - Anti-NLRP3 antibody [EPR23094-1] (ab263899)

ELISA analysis of NLRP3 recombinant protein at 1000 ng/mL with ab263899. An Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit lgG (H+L) at 1/2500 dilution was used as the secondary antibody.



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