abcam

ab256303

Anti-Caspase-4 antibody [EPR20921-83] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR20921-83
Purification technique	Affinity purification Protein A
Concentration	1.076 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Not recommended

Species	Human
Dilution info	-

Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Human
Dilution info	-
Notes	-

ICC/IF

Not recommended

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Target data

See full target information CASP4

Function

Inflammatory caspase that acts as the effector of the non-canonical inflammasome by mediating lipopolysaccharide (LPS)-induced pyroptosis (PubMed:25119034, PubMed:26375003, PubMed:32109412, PubMed:34671164, PubMed:37001519, PubMed:37993712, PubMed:37993714). Also indirectly activates the NLRP3 and NLRP6 inflammasomes (PubMed:23516580, PubMed:26375003, PubMed:32109412, PubMed:7797510). Acts as a thiol protease that cleaves a tetrapeptide after an Asp residue at position P1: catalyzes cleavage of CGAS, GSDMD and IL18 (PubMed:15326478, PubMed:23516580, PubMed:26375003, PubMed:28314590, PubMed:32109412, PubMed:37993712, PubMed:37993714, PubMed:7797510). Effector of the non-canonical inflammasome independently of NLRP3 inflammasome and CASP1: the non-canonical inflammasome promotes pyroptosis through GSDMD cleavage without involving secretion of cytokine IL1B (PubMed:25119034, PubMed:25121752, PubMed:26375003, PubMed:31268602, PubMed:32109412, PubMed:37993712, PubMed:37993714). In the non-canonical inflammasome, CASP4 is activated by direct binding to the lipid A moiety of LPS without the need of an upstream sensor (PubMed:25119034, PubMed:25121752, PubMed:29520027, PubMed:32510692, PubMed:32581219, PubMed:37993712). LPS-binding promotes CASP4 activation and CASP4-mediated cleavage of GSDMD and IL18, followed by IL18 secretion through the GSDMD pore, pyroptosis of infected cells and their extrusion into the gut lumen (PubMed:25119034, PubMed:25121752, PubMed:37993712, PubMed:37993714). Also indirectly promotes secretion of mature cytokines (IL1A and HMGB1) downstream of GSDMD-mediated pyroptosis via activation of the NLRP3 and NLRP6 inflammasomes (PubMed:26375003, PubMed:32109412). Involved in NLRP3-dependent CASP1 activation and IL1B secretion in response to non-canonical activators, such as UVB radiation or cholera enterotoxin (PubMed:22246630, PubMed:23516580, PubMed:24879791, PubMed:25964352, PubMed:26173988, PubMed:26174085, PubMed:26508369). Involved in NLRP6 inflammasome-dependent activation in response to lipoteichoic acid (LTA), a cell-wall component of Gram-positive bacteria, which leads to CASP1 activation and IL1B secretion (PubMed:33377178). Involved in LPS-induced IL6 secretion; this activity may not require caspase enzymatic activity (PubMed:26508369). The non-canonical inflammasome is required for innate immunity to cytosolic, but not vacuolar, bacteria (By similarity). Plays a crucial role in the restriction of S.typhimurium replication in colonic epithelial cells during infection (PubMed:25121752, PubMed:25964352). Activation of the non-canonical inflammasome in brain endothelial cells can lead to excessive pyroptosis, leading to blood-brain barrier breakdown (By similarity). Pyroptosis limits bacterial replication, while cytokine secretion promotes the recruitment and activation of immune cells and triggers mucosal inflammation (PubMed:25121752, PubMed:25964352, PubMed:26375003). May also act as an activator of adaptive immunity in dendritic cells, following activation by oxidized phospholipid 1-palmitoyl-2-arachidonoyl- sn-glycero-3-phosphorylcholine, an oxidized phospholipid (oxPAPC) (By similarity). Involved in cell death induced by endoplasmic reticulum stress and by treatment with cytotoxic APP peptides found in Alzheimer's patient brains (PubMed:15123740, PubMed:22246630, PubMed:23661706). Cleavage of GSDMD is not strictly dependent on the consensus cleavage site but depends on an exosite interface on CASP4 that recognizes and binds the Gasdermin-D, C-terminal (GSDMD-CT) part (PubMed:32109412). Catalyzes cleavage and maturation of IL18; IL18 processing also depends of the exosite interface on CASP4 (PubMed:15326478, PubMed:37993712, PubMed:37993714). In contrast, it does not directly process IL1B (PubMed:7743998, PubMed:7797510, PubMed:7797592). During non-canonical inflammasome activation, cuts CGAS and may play a role in the

regulation of antiviral innate immune activation (PubMed:28314590). (Microbial infection) In response to the Td92 surface protein of the periodontal pathogen T.denticola, activated by cathepsin CTSG which leads to production and secretion of IL1A and pyroptosis of gingival fibroblasts.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab256303 is the carrier-free version of ab238124.

This antibody was developed in collaboration with Dr Feng Shao's lab, NIBS.

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

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

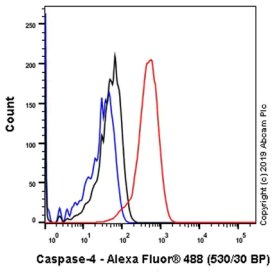
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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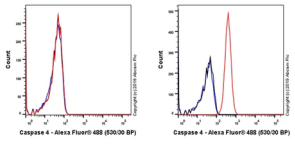
3 product images



Flow Cytometry (Intracellular) - Anti-Caspase-4 antibody [EPR20921-83] - BSA and Azide free (ab256303)

Caspase-4 Flow Cytometry (Intracellular) staining using rabbit Anti-Caspase-4 antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized IM-9 (human multiple myeloma B Lymphoblast) cell line labeling Caspase 4 with ab238124 at 1/60 (red) compared with a Rabbit monoclonal IgG (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti rabbit IgG (Alexa Fluor® 488, ab150097), at 1/5000 dilution was used as the secondary antibody.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab238124).

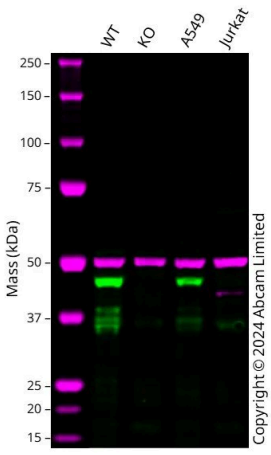


Flow Cytometry (Intracellular) - Anti-Caspase-4 antibody [EPR20921-83] - BSA and Azide free (ab256303)

Caspase-4 Flow Cytometry (Intracellular) staining using rabbit Anti-Caspase-4 antibody

Intracellular flow cytometric analysis of THP-1 (Human monocytic leukemia monocyte) cells labeling Caspase-4 with ab238124 at 1/60 (right panel, red) compared with HEK-293T (Human

epithelial cell line from embryonic kidney transformed with large T antigen) cells (left panel, red). Goat anti rabbit IgG (Alexa Fluor® 488, ab150097), at 1/5000 dilution was used as the secondary antibody. Rabbit monoclonal IgG (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). **Negative control**: HEK-293T (PMID: 25119034).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab238124).



Western blot - Anti-Caspase-4 antibody [EPR20921-83] - BSA and Azide free (ab256303)

Caspase-4 Western blot staining using rabbit Anti-Caspase-4 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab238124).

Western blot: Anti-CASP4 antibody [EPR20921-83] (ab238124) staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab238124 was shown to bind specifically to CASP4. A band was observed at 43 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in CASP4 knockout cell line. To generate this image, wild-type and CASP4 knockout HCT 116 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) 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 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-Caspase-4 antibody [EPR20921-83] (ab238124) at 1/1000 dilution

Lane 1:
Wild-type HCT 116 cell lysate at 20 µg

Lane 2:
CASP4 knockout HCT 116 cell lysate at 20 µg

Lane 3:
A549 cell lysate at 20 µg

Lane 4:
Jurkat cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 43 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.