abcam

Product datasheet

Anti-IL-1RAcP antibody [EPR22678-67] ab256461



Recombinant RabMAb

2 References 7 Images

Overview

Product name Anti-IL-1RAcP antibody [EPR22678-67]

Description Rabbit monoclonal [EPR22678-67] to IL-1RAcP

Host species Rabbit

Tested applications Suitable for: WB, Flow Cyt, IP, Indirect ELISA

Unsuitable for: ICC/IF or IHC-P

Reacts with: Human Species reactivity

Recombinant fragment. This information is proprietary to Abcam and/or its suppliers. **Immunogen**

Positive control WB: KARPAS-299 and HepG2 whole cell lysate. Flow Cyt: HeLa and HepG2 cells. IP: KARPAS-

299 whole cell lysate.

General notes 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**.

Properties

Form Liquid

Storage instructions 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.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR22678-67

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab256461 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 80 kDa (predicted molecular weight: 65 kDa).
Flow Cyt		1/60.
IP		1/30.
Indirect ELISA		Use at an assay dependent concentration.

Application notes

Is unsuitable for ICC/IF or IHC-P.

Target

Function

Coreceptor with IL1R1. Associates with IL1R1 bound to IL1B to form the high affinity interleukin-1 receptor complex which mediates interleukin-1-dependent activation of NF-kappa-B and other pathways. Signaling involves the recruitment of adapter molecules such as TOLLIP, MYD88, and IRAK1 or IRAK2 via the respective TIR domains of the receptor/coreceptor subunits. Recruits TOLLIP to the signaling complex. Does not bind to interleukin-1 alone; binding of IL1RN to IL1R1, prevents its association with IL1R1 to form a signaling complex. The cellular response is modulated through a non-signaling association with the membrane IL1R2 decoy receptor. Secreted forms (isoforms 2 and 3) associate with secreted ligand-bound IL1R2 and increase the affinity of secreted IL1R2 for IL1B; this complex formation may be the dominant mechanism for neutralization of IL1B by secreted/soluble receptors.

Tissue specificity

Detected in liver, skin, placenta, thymus and lung.

Sequence similarities

Belongs to the interleukin-1 receptor family.

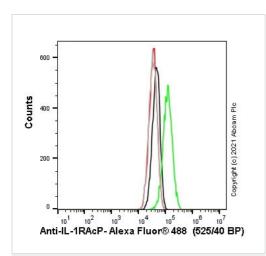
Contains 3 lg-like C2-type (immunoglobulin-like) domains.

Contains 1 TIR domain.

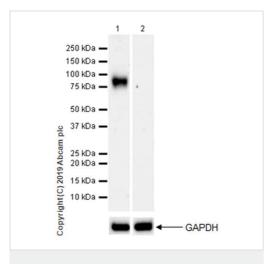
Cellular localization

Secreted and Cell membrane.

Images



Flow Cytometry - Anti-IL-1RAcP antibody [EPR22678-67] (ab256461)



Western blot - Anti-IL-1RAcP antibody [EPR22678-67] (ab256461)

Flow cytometry overlay histogram showing wild-type HeLa (green line) and IL1RAP knockout HeLa cells (ab273375) stained with ab256461 (red line). The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab256461) (1x10 6 in 100 μ l at 10 μ /ml) for 30 min at 4°C.

The secondary antibody Goat anti-rabbit lgG H&L (Alexa Fluor[®] 488, pre-adsorbed) (<u>ab150081</u>) was used at 1/2000 for 30 min at 4°C.

Isotype control antibody was Rabbit IgG (monoclonal) (<u>ab172730</u>) used at the same concentration and conditions as the primary antibody (wild-type HeLa - black line; IL1RAP knockout HeLa - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

All lanes : Anti-IL-1RAcP antibody [EPR22678-67] (ab256461) at 1/1000 dilution

Lane 1: KARPAS-299 (human anaplastic large cell lymphoma) whole cell lysate

Lane 2 : Raji (human burkitt's lymphoma b lymphocyte) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 65 kDa

Exposure time: 3 minutes

The molecular weight observed is consistent with what has been described in the literature (PMID: 30514753).

Negative control: Raji (PMID: 30514753).

Blocking/Dilution buffer: 5% NFDM/TBST.

Anti-IL-1RAcP antibody [EPR22678-67] (ab256461) at 1/1000 dilution + HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate at 20 µg



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

Predicted band size: 65 kDa

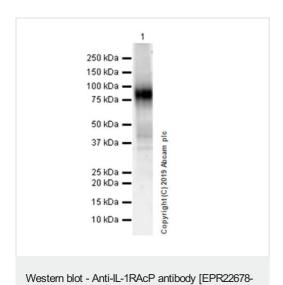
Exposure time: 3 minutes

The molecular weight observed is consistent with what has been described in the literature (PMID: 30514753).

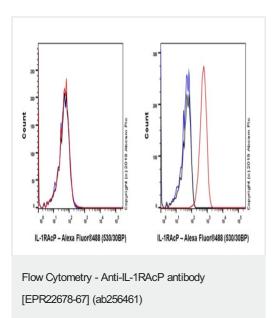
This blot was developed using a higher sensitivity ECL substrate.

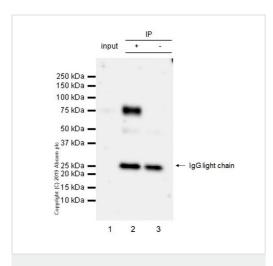
Blocking/Dilution buffer: 5% NFDM/TBT.

Flow cytometric analysis of Raji (Human burkitt's lymphoma b lymphocyte, Left panel) and HepG2 (Human hepatocellular carcinoma epithelial cell, Right panel) cells labeling IL-1RAcP with ab256461 at a 1/60 dilution (1µg, 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). Goat anti rabbit IgG (Alexa Fluor[®] 488, ab150077) at a 1/2000 dilution was used as the secondary antibody. **Negative control:** Raji (PMID: 30514753). Gated on viable cells.

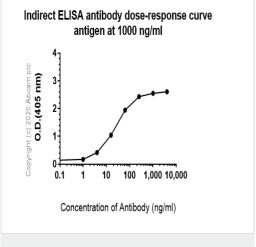


67] (ab256461)





Immunoprecipitation - Anti-IL-1RAcP antibody [EPR22678-67] (ab256461)



Indirect ELISA - Anti-IL-1RAcP antibody [EPR22678-67] (ab256461)

lL-1RAcP was immunoprecipitated from 0.35 mg KARPAS-299 (human anaplastic large cell lymphoma) whole cell lysate 10 μ g with ab256461 at 1/30 dilution (2 μ g in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab256461 at a 1/1000 dilution (0.59 μ g/ml). VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.

Lane 1: KARPAS-299 (human anaplastic large cell lymphoma) whole cell lysate 10µg.

Lane 2: ab256461 IP in KARPAS-299 whole cell lysate.

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab256461 in KARPAS-299 whole cell lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 75 secs.

ELISA analysis of Hu IL1RAP recombinant protein at 1000 ng/mL with ab256461. 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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