abcam

Product datasheet

Anti-IRAK-1 antibody ab85071

1 Abreviews 3 Images

Overview

Product name	Anti-IRAK-1 antibody
Description	Rabbit polyclonal to IRAK-1
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat, Cow, Macaque monkey
Immunogen	Synthetic peptide conjugated to KLH derived from within residues 350 - 450 of Human IRAK.Read Abcam's proprietary immunogen policy(Peptide available as ab99364.)
Positive control	Purchase matching WB positive control: **Recombinant Human IRAK-1 protein** This antibody gave a positive signal in Human liver tissue lysate.

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.02% Sodium Azide Constituents: 1% BSA, PBS, pH 7.4
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab85071** 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	★★★☆☆	Use a concentration of 1 µg/ml. Detects a band of approximately 74 kDa (predicted molecular weight: 76 kDa).
ICC/IF		Use a concentration of 5 µg/ml.

Target

Function

Binds to the IL-1 type I receptor following IL-1 engagement, triggering intracellular signaling cascades leading to transcriptional up-regulation and mRNA stabilization. Isoform 1 binds rapidly but is then degraded allowing isoform 2 to mediate a slower, more sustained response to the cytokine. Isoform 2 is inactive suggesting that the kinase activity of this enzyme is not required for IL-1 signaling. Once phosphorylated, IRAK1 recruits the adapter protein PELI1.

Tissue specificity

Isoform 1 and isoform 2 are ubiquitously expressed in all tissues examined, with isoform 1 being more strongly expressed than isoform 2.

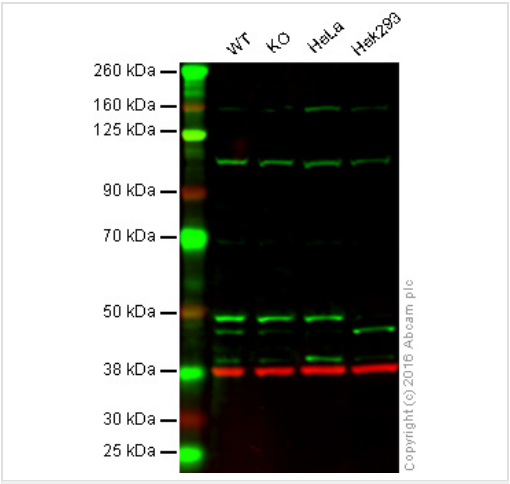
Sequence similarities

Belongs to the protein kinase superfamily. TKL Ser/Thr protein kinase family. Pelle subfamily. Contains 1 protein kinase domain.

Post-translational modifications

Autophosphorylated or is transphosphorylated by IRAK4 following recruitment to the IL-1RI. In the case of isoform 1, this is linked to ubiquitination and degradation.
Polyubiquitinated; after cell stimulation with IL-1-beta. Polyubiquitination occurs with polyubiquitin chains linked through 'Lys-63'.

Images



Western blot - Anti-IRAK antibody (ab85071)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)

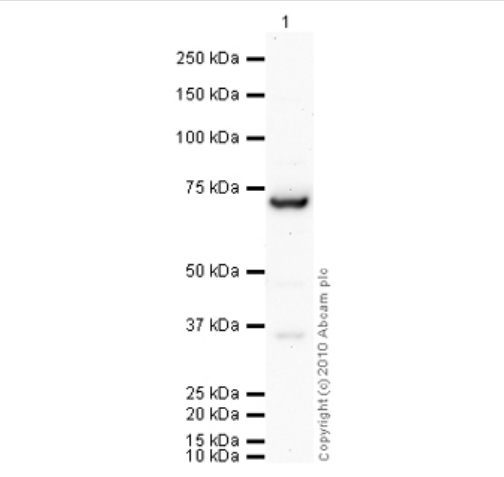
Lane 2: IRAK1 knockout HAP1 whole cell lysate (20 µg)

Lane 3: HeLa whole cell lysate (20 µg)

Lane 4: Hek293 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab85071 observed at 85 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab85071 was shown not to specifically react with IRAK1 when IRAK1 knockout samples were used. Wild-type and IRAK1 knockout samples were subjected to SDS-PAGE. Ab85071 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1 ug/ml and 1/10000 dilution respectively. Blots were developed with 800CW Goat anti Rabbit and 680CW Goat anti Mouse secondary antibodies at 1/10012 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-IRAK antibody (ab85071)

Anti-IRAK-1 antibody (ab85071) at 1 µg/ml + Human liver tissue lysate - total protein (ab29889) at 10 µg

Secondary

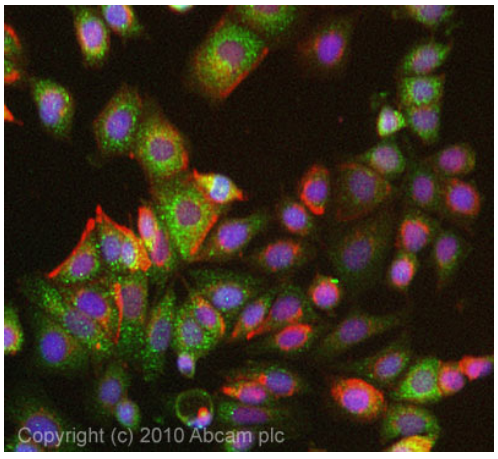
Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 76 kDa
Observed band size: 74 kDa
why is the actual band size different from the predicted?
Additional bands at: 36 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 3 minutes



Immunocytochemistry/ Immunofluorescence - Anti-IRAK antibody (ab85071)

ICC/IF image of ab85071 stained MCF-7 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab85071 at 5µg/ml overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti- rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM. This antibody also gave a positive result in Methanol fixed (100%, 5min) MCF-7 cells at 5ug/ml

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