# abcam

## Product datasheet

## Anti-IRAK4 antibody [2H9] ab119942

## KO VALIDATED

6 References 8 Images

#### Overview

Product name Anti-IRAK4 antibody [2H9]

**Description** Mouse monoclonal [2H9] to IRAK4

Host species Mouse

Tested applications Suitable for: WB, ELISA, IHC-P, Flow Cyt, ICC/IF

Species reactivity

Reacts with: Mouse, Human, African green monkey, Recombinant fragment

Recombinant fragment, corresponding to amino acids 21-198 of Human IRAK4

Positive control Recombinant IRAK4 protein; THP-1, Hela, K562, MCF7, U-87 MG, RAW264.7, Jurkat and Cos7

cell lysates; Human lung cancer amd kidney cancer tissues; Hela cells This antibody gave a

positive result in IF in the following Formaldehyde fixed cell line: HepG2.

**General notes**The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

## **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at -20°C long term.

**Storage buffer** Preservative: 0.05% Sodium azide

Constituent: PBS

**Purity** Protein G purified

**Purification notes** Purified from tissue culture supernatant.

**Clonality** Monoclonal

Clone number 2H9
Isotype IgG1

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## **Applications**

#### The Abpromise guarantee

Our Abpromise quarantee covers the use of ab119942 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		1/500 - 1/2000. Predicted molecular weight: 52 kDa.
ELISA		1/10000.
IHC-P		1/200 - 1/1000.
Flow Cyt		1/200 - 1/400.  ab170190 - Mouse monoclonal lgG1, is suitable for use as an isotype control with this antibody.
ICC/IF		1/100.

#### **Target**

**Function** 

Required for the efficient recruitment of IRAK1 to the IL-1 receptor complex following IL-1 engagement, triggering intracellular signaling cascades leading to transcriptional up-regulation and mRNA stabilization. Phosphorylates IRAK1.

Involvement in disease

Defects in IRAK4 are the cause of recurrent isolated invasive pneumococcal disease type 1 (IPD1) [MIM:610799]. Recurrent invasive pneumococcal disease (IPD) is defined as two episodes of IPD occurring at least 1 month apart, whether caused by the same or different serotypes or strains. Recurrent IPD occurs in at least 2% of patients in most series, making IPD the most important known risk factor for subsequent IPD.

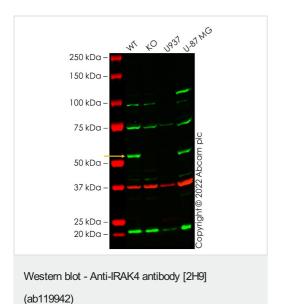
Defects in IRAK4 are the cause of IRAK4 deficiency (IRAK4D) [MIM:607676]. IRAK4 deficiency causes extracellular pyogenic bacterial and fungal infections in otherwise healthy children.

Sequence similarities

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

Contains 1 protein kinase domain.

## **Images**



All lanes: Anti-IRAK4 antibody [2H9] (ab119942) at 1/500 dilution

Lane 1: Wild-type THP-1 cell lysate

Lane 2: IRAK4 knockout THP-1 cell lysate

Lane 3: U937 cell lysate

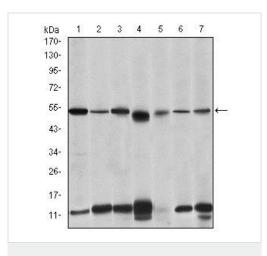
Lane 4: U-87 MG cell lysate

Lysates/proteins at 20 µg per lane.

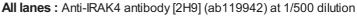
Performed under reducing conditions.

**Predicted band size:** 52 kDa **Observed band size:** 55 kDa

False colour image of Western blot: Anti-IRAK4 antibody [2H9] staining at 1/500 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (ab181602) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab119942 was shown to bind specifically to IRAK4. A band was observed at 55 kDa in wild-type THP-1 cell lysates with no signal observed at this size in IRAK4 knockout cell line ab281630 (knockout cell lysate ab282980). To generate this image, wild-type and IRAK4 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 3 % milk in TBS-0.1 % Tween<sup>®</sup> 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-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216772) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (ab216777) at 1/20000 dilution.



Western blot - Anti-IRAK4 antibody [2H9] (ab119942)



Lane 1 : THP-1 cell lysate

Lane 2 : HeLa cell lysate

Lane 3: K562 cell lysate

Lane 4: MCF7 cell lysate

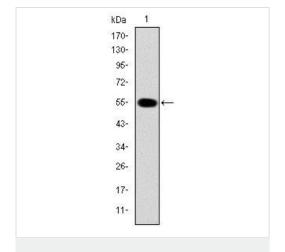
Lane 5: RAW264.7 cell lysate

Lane 6: Jurkat cell lysate

Lane 7: COS-7 (African green monkey kidney fibroblast-like cell

line) cell lysate

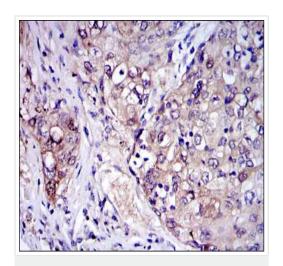
Predicted band size: 52 kDa



Western blot - Anti-IRAK4 antibody [2H9] (ab119942)

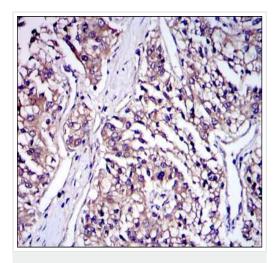
Anti-IRAK4 antibody [2H9] (ab119942) at 1/500 dilution + Recombinant IRAK4 protein

Predicted band size: 52 kDa



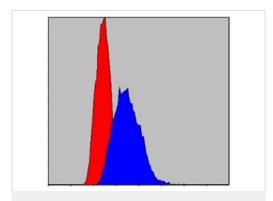
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IRAK4 antibody [2H9] (ab119942)

ab119942, at 1/200 dilution, staining IRAK4 in paraffin-embedded Human lung cancer tissue by Immunohistochemistry with DAB staining.



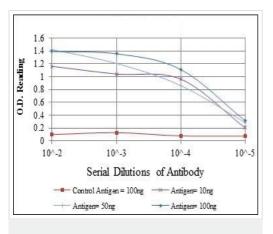
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IRAK4 antibody [2H9] (ab119942)

ab119942, at 1/200 dilution, staining IRAK4 in paraffin-embedded Human kidney cancer tissue by Immunohistochemistry with DAB staining.



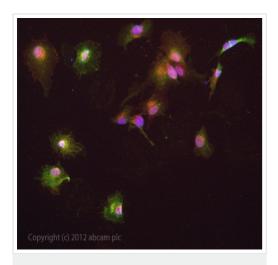
Flow Cytometry - Anti-IRAK4 antibody [2H9] (ab119942)

Flow cytometric analysis of Hela cells using ab119942 at 1/200 dilution (blue) and negative control (red).



ELISA - Anti-IRAK4 antibody [2H9] (ab119942)

ELISA using ab119942.



Immunocytochemistry/ Immunofluorescence - Anti-IRAK4 antibody [2H9] (ab119942)

ab119942 stained HepG2 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 ab119942 at 1/100 dilution overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- mouse (ab96879) lgG (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.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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