

# Anti-IRAK-1 Antibody

## HA500377



<b>Product Type:</b>	Rabbit polyclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human, Mouse
<b>Applications:</b>	WB, IHC-P, IF-Cell
<b>Molecular Wt:</b>	Predicted band size: 77 kDa

**Description:** Serine/threonine-protein kinase that plays a critical role in initiating innate immune response against foreign pathogens. Involved in Toll-like receptor (TLR) and IL-1R signaling pathways. Is rapidly recruited by MYD88 to the receptor-signaling complex upon TLR activation. Association with MYD88 leads to IRAK1 phosphorylation by IRAK4 and subsequent autophosphorylation and kinase activation. Phosphorylates E3 ubiquitin ligases Pellino proteins (PELI1, PELI2 and PELI3) to promote pellino-mediated polyubiquitination of IRAK1. Then, the ubiquitin-binding domain of IKBKG/NEMO binds to polyubiquitinated IRAK1 bringing together the IRAK1-MAP3K7/TAK1-TRAF6 complex and the NEMO-IKKA-IKKB complex. In turn, MAP3K7/TAK1 activates IKKs (CHUK/IKKA and IKBKB/IKKB) leading to NF-kappa-B nuclear translocation and activation. Alternatively, phosphorylates TIRAP to promote its ubiquitination and subsequent degradation. Phosphorylates the interferon regulatory factor 7 (IRF7) to induce its activation and translocation to the nucleus, resulting in transcriptional activation of type I IFN genes, which drive the cell in an antiviral state. When sumoylated, translocates to the nucleus and phosphorylates STAT3. Isoform 1 and isoform 2 are ubiquitously expressed in all tissues examined, with isoform 1 being more strongly expressed than isoform 2.

<b>Immunogen:</b>	Recombinant protein within human IRAK-1 aa 1-250.
<b>Positive control:</b>	293T cell lysates, rat brain tissue, human breast tissue, mouse large intestine tissue, Hela, PC-3M.
<b>Subcellular location:</b>	Cytoplasm, Nucleus.
<b>Database links:</b>	SwissProt: P51617 Human   Q62406 Mouse
<b>Recommended Dilutions:</b>	
<b>WB</b>	1:2,000
<b>IHC-P</b>	1:400-1:800
<b>Storage Buffer:</b>	PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
<b>Storage Instruction:</b>	Shipped at 4°C. Store at +4°C short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20°C long term.
<b>Purity:</b>	Immunogen affinity purified.

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Orders: 0086-571-88062880

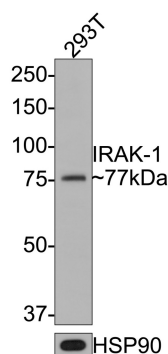
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Applications: WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

## Images



**Fig1:** Western blot analysis of IRAK-1 on 293T cell lysates with Rabbit anti-IRAK-1 antibody (HA500377) at 1/2,000 dilution.

Lysates/proteins at 10 µg/Lane.

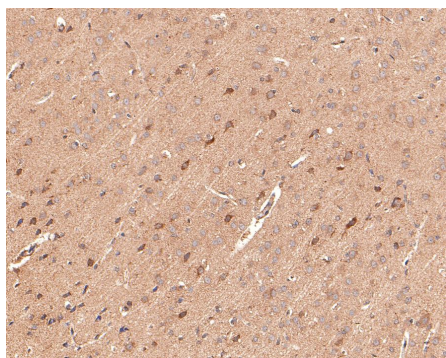
Predicted band size: 77 kDa

Observed band size: 77 kDa

Exposure time: 1 minute;

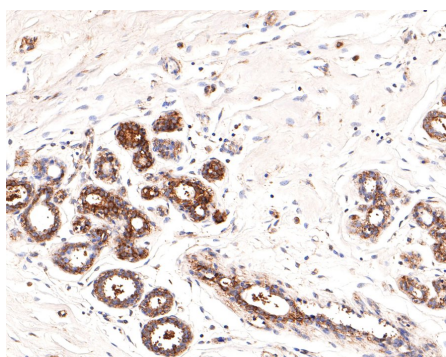
8% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA500377) at 1/2,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.



**Fig2:** Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-IRAK-1 antibody (HA500377) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA500377) at 1/400 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig3:** Immunohistochemical analysis of paraffin-embedded human breast tissue with Rabbit anti-IRAK-1 antibody (HA500377) at 1/800 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA500377) at 1/800 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

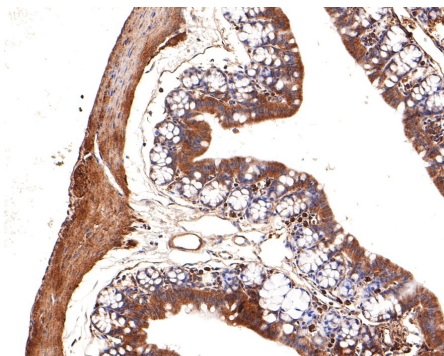
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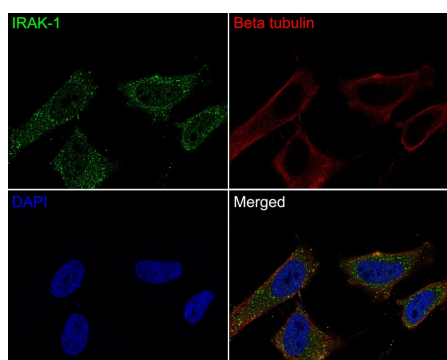
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**Fig4:** Immunohistochemical analysis of paraffin-embedded mouse large intestine tissue with Rabbit anti-IRAK-1 antibody (HA500377) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA500377) at 1/400 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

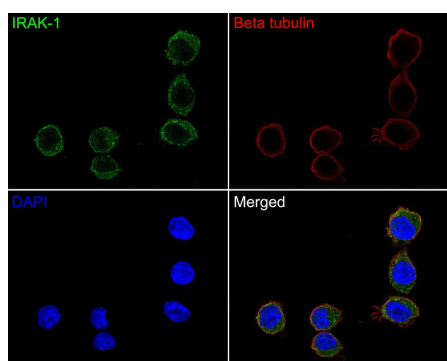
**Fig5:** Immunocytochemistry analysis of Hela cells labeling IRAK-1 with Rabbit anti-IRAK-1 antibody (HA500377) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 °C, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-IRAK-1 antibody (HA500377) at 1/100 dilution in 2% negative goat serum overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4°C. Goat Anti-Mouse IgG H&L (iFluor™ 647, HA1127) were used as the secondary antibody at 1/1,000 dilution.

**Fig6:** Immunocytochemistry analysis of PC-3M cells labeling IRAK-1 with Rabbit anti-IRAK-1 antibody (HA500377) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 °C, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-IRAK-1 antibody (HA500377) at 1/100 dilution in 2% negative goat serum overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4°C. Goat Anti-Mouse IgG H&L (iFluor™ 647, HA1127) were used as the secondary antibody at 1/1,000 dilution.

**Note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

### Background References

1. Dunne A., Carpenter S., Brikos C., Gray P., Strelow A., Wesche H., Morrice N., O'Neill L.A. IRAK1 and IRAK4 promote phosphorylation, ubiquitination, and degradation of MyD88 adaptor-like (Mal). J. Biol. Chem. 285:18276-18282(2010).
2. Ordureau A., Smith H., Windheim M., Peggie M., Carrick E., Morrice N., Cohen P. The IRAK-catalysed activation of the E3 ligase function of Pellino isoforms induces the Lys63-linked polyubiquitination of IRAK1. Biochem. J. 409:43-52(2008).

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