Anti-IRG1 Antibody [PSH07-31]

HA722819

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Mouse
Applications:	WB, IF-Cell
Molecular Wt:	Predicted band size: 54 kDa
Clone number:	PSH07-31

Description: Cis-aconitate decarboxylase that catalyzes production of itaconate and is involved in the inhibition of the inflammatory response. Acts as a negative regulator of the Toll-like receptors (TLRs)-mediated inflammatory innate response by stimulating the tumor necrosis factor alpha-induced protein TNFAIP3 expression via reactive oxygen species (ROS) in LPS-tolerized macrophages. Involved in antimicrobial response of innate immune cells; ACOD1-mediated itaconic acid production contributes to the antimicrobial activity of macrophages by generating itaconate, leading to alkylation of proteins, such as TFEB. Involved in antiviral response following infection by flavivirus in neurons: ACOD1-mediated itaconate production inhibits the activity of succinate dehydrogenase, generating a metabolic state in neurons that suppresses replication of viral genomes (By similarity). Plays a role in the embryo implantation (By similarity).

Immunogen: Recombinant protein within mouse IRG1 aa 1-488.

Positive control: RAW264.7 treated with 0.1μg/mL LPS for 6 hours cell lysate, J774A.1 treated with 1μg/mL LPS for 24 hours cell lysate, mBMMC treated with 0.1μg/mL LPS and 10ng/mL IFN-gamma for 24 hours cell lysate, mBMSC treated with 40ng/mL MCSF for 7 days then add 100ng/mL LPS for 48 hours cell lysate, RAW264.7 cells treated with 0.1μg/mL LPS for 6 hours.

Subcellular location: Mitochondrion.

Database links: SwissProt: P54987 Mouse

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

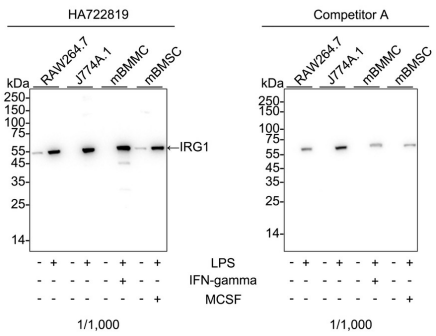

Fig1: Western blot analysis of IRG1 on different lysates with Rabbit anti-IRG1 antibody (HA722819) at 1/1,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: RAW264.7 cell lysate
Lane 2: RAW264.7 treated with 0.1μg/mL LPS for 6 hours cell lysate
Lane 3: J774A.1 cell lysate
Lane 4: J774A.1 treated with 1μg/mL LPS for 24 hours cell lysate
Lane 5: mBMMC cell lysate
Lane 6: mBMMC treated with 0.1μg/mL LPS and 10ng/mL IFN-gamma for 24 hours cell lysate
Lane 7: mBMSC cell lysate
Lane 8: mBMSC treated with 40ng/mL MCSF for 7 days then add 100ng/mL LPS for 48 hours cell lysate

Lysates/proteins at 10 μg/Lane.

Predicted band size: 54 kDa
Observed band size: 54 kDa

Exposure time: 10 seconds; ECL: K1801;

4-20% 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 (HA722819) at 1/1,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

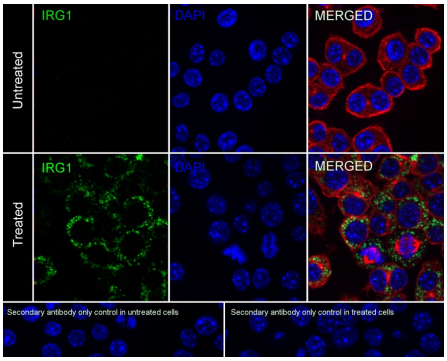

Fig2: Immunocytochemistry analysis of RAW264.7 cells treated with 0.1μg/mL LPS for 6 hours labeling IRG1 with Rabbit anti-IRG1 antibody (HA722819) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-IRG1 antibody (HA722819) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Background References

1. Wu R et al. The Dual Role of ACOD1 in Inflammation. J Immunol. 2023 Aug
2. Wu R et al. Mitochondrial ACOD1/IRG1 in infection and sterile inflammation. J Intensive Med. 2022 Feb