

Anti-IRF8 Antibody [PSH10-05]

HA723174



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 48 kDa
Clone number:	PSH10-05

Description: Interferon regulatory factor 8 (IRF8) also known as interferon consensus sequence-binding protein (ICSBP), is a protein that in humans is encoded by the IRF8 gene. IRF8 is a transcription factor that plays critical roles in the regulation of lineage commitment and in myeloid cell maturation including the decision for a common myeloid progenitor (CMP) to differentiate into a monocyte precursor cell. Interferon Consensus Sequence-binding protein (ICSBP) is a transcription factor of the interferon regulatory factor (IRF) family. Proteins of this family are composed of a conserved DNA-binding domain in the N-terminal region and a divergent C-terminal region that serves as the regulatory domain. The IRF family proteins bind to the IFN-stimulated response element (ISRE) and regulate expression of genes stimulated by type I IFNs, namely IFN- α and IFN- β . IRF family proteins also control expression of IFN- α and IFN- β -regulated genes that are induced by viral infection.

Immunogen: Recombinant protein within human IRF8 aa 81-426.

Positive control: Raji cell lysate, Ramos cell lysate, THP-1 cell lysate, A20 cell lysate, A20, THP-1.

Subcellular location: Nucleus, Cytoplasm.

Database links: SwissProt: Q02556 Human | P23611 Mouse

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100
FC	1:1,000
IP	1-2 μ g/sample

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

Storage Instruction: Shipped at 4 $^{\circ}$ C. Store at +4 $^{\circ}$ C short term (1-2 weeks). Store at -20 $^{\circ}$ C long term.

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders: 0086-571-88062880

Technical: 0086-571-89986345

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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

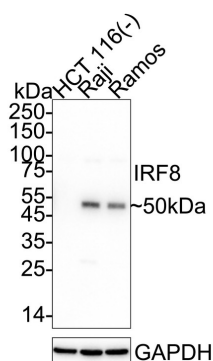
Images

Fig1: Western blot analysis of IRF8 on different lysates with Rabbit anti-IRF8 antibody (HA723174) at 1/2,000 dilution.

Lane 1: HCT 116 cell lysate (negative)

Lane 2: Raji cell lysate

Lane 3: Ramos cell lysate



Lysates/proteins at 20 µg/Lane.

Predicted band size: 48 kDa

Observed band size: 50 kDa

Exposure time: 28 seconds; ECL: K1802;

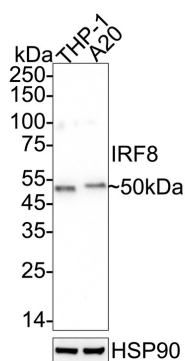
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA723174) at 1/2,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig2: Western blot analysis of IRF8 on different lysates with Rabbit anti-IRF8 antibody (HA723174) at 1/2,000 dilution.

Lane 1: THP-1 cell lysate

Lane 2: A20 cell lysate



Lysates/proteins at 20 µg/Lane.

Predicted band size: 48 kDa

Observed band size: 50 kDa

Exposure time: 3 minutes; 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 (HA723174) at 1/2,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

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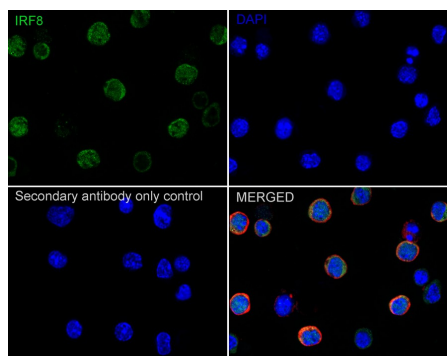
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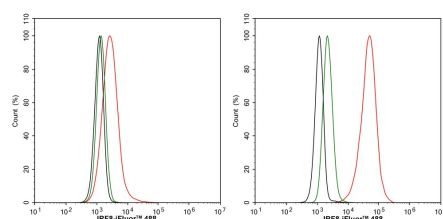
Fig3: Immunocytochemistry analysis of A20 cells labeling IRF8 with Rabbit anti-IRF8 antibody (HA723174) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-IRF8 antibody (HA723174) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. 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 (HA601187, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig4: Flow cytometric analysis of HCT 116 (left, negative) and THP-1 (right, positive) cells labeling IRF8.



Cells were fixed and permeabilized. Then stained with the primary antibody (HA723174, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4 °C for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4 °C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

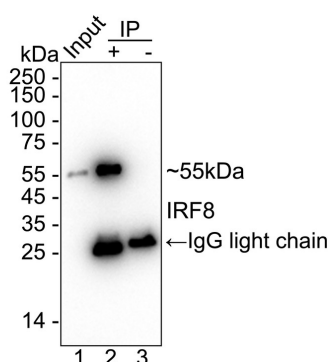


Fig5: IRF8 was immunoprecipitated from 0.2 mg THP-1 cell lysate with HA723174 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA723174 at 1/1,000 dilution. Mouse Anti-Rabbit IgG kappa light chain secondary antibody (M1208-2) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: THP-1 cell lysate (input)
Lane 2: HA723174 IP in THP-1 cell lysate
Lane 3: Rabbit IgG instead of HA723174 in THP-1 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 59 seconds; ECL: K1801

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

Background References

1. Nixon BG et al. Tumor-associated macrophages expressing the transcription factor IRF8 promote T cell exhaustion in cancer. *Immunity*. 2022 Nov
2. Moorman HR et al. IRF8: Mechanism of Action and Health Implications. *Cells*. 2022 Aug

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