Anti-F4/80 Antibody [PSH0-85]

HA721520

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Mouse, Rat
Applications:	WB, IHC-P, IF-Tissue, IHC-Fr, mIHC
Molecular Wt:	Predicted band size: 102 kDa
Clone number:	PSH0-85

Description: EGF-like module-containing mucin-like hormone receptor-like 1 also known as F4/80 is a protein encoded by the ADGRE1 gene. EMR1 is a member of the adhesion GPCR family. Adhesion GPCRs are characterized by an extended extracellular region often possessing N-terminal protein modules that is linked to a TM7 region via a domain known as the GPCR-Autoproteolysis INducing (GAIN) domain. EMR1 expression in human is restricted to eosinophils and is a specific marker for these cells. The murine homolog of EMR1, F4/80, is a well-known and widely used marker of murine macrophage populations. The N-terminal fragment (NTF) of EMR1 contains 4-6 Epidermal Growth Factor-like (EGF-like) domains in human and 4-7 EGF-like domains in the mouse. Utilizing F4/80 knockout mice, Lin et al. showed that F4/80 is not necessary for the development of tissue macrophages but is required for the induction of efferent CD8+ regulatory T cells needed for peripheral tolerance.

Immunogen: Recombinant protein within mouse F4/80 aa 1-650 / 931.

Positive control: RAW264.7 cell lysate, rat spleen tissue lysate, mouse liver tissue, mouse spleen tissue, rat liver tissue, rat spleen tissue.

Subcellular location: Cell membrane.

Database links: SwissProt: Q61549 Mouse | Q5Y4N8 Rat

Recommended Dilutions:

WB	1:1,000
IHC-P	1:200-1:1,000
IF-Tissue	1:50-1:500
IHC-Fr	1:200
mIHC	1:500

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

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

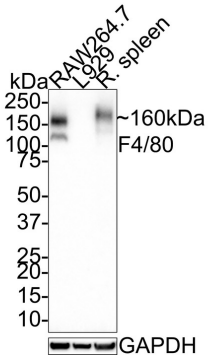

Fig1: Western blot analysis of F4/80 on different lysates with Rabbit anti-F4/80 antibody (HA721520) at 1/1,000 dilution.

Lane 1: RAW264.7 cell lysate (no heat) (20 µg/Lane)
Lane 2: L929 cell lysate (no heat) (negative) (20 µg/Lane)
Lane 3: Rat spleen tissue lysate (70℃ heat) (40 µg/Lane)

Predicted band size: 102 kDa
Observed band size: 160 kDa

Exposure time: 2 minutes;

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 (HA721520) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

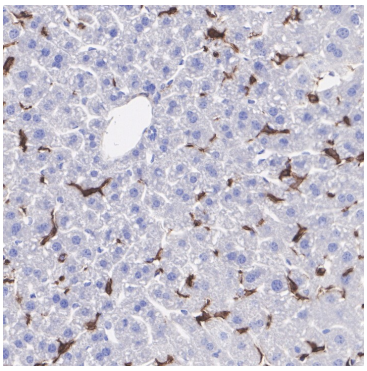

Fig2: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-F4/80 antibody (HA721520) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA721520) at 1/1,000 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.

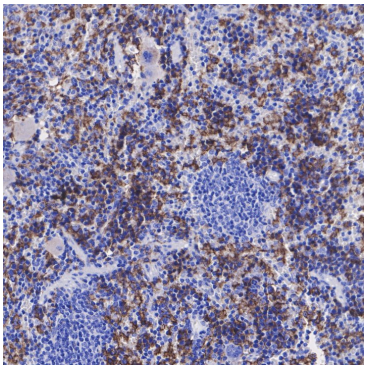

Fig3: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-F4/80 antibody (HA721520) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA721520) at 1/1,000 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.

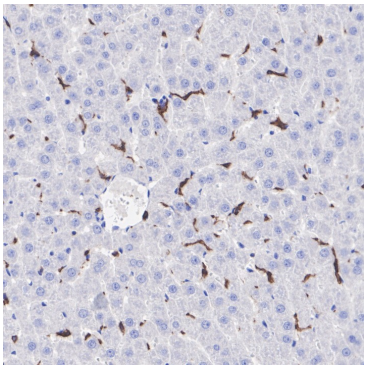

Fig4: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-F4/80 antibody (HA721520) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA721520) at 1/1,000 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.

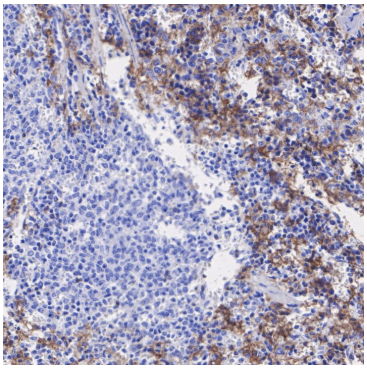

Fig5: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-F4/80 antibody (HA721520) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA721520) at 1/1,000 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.

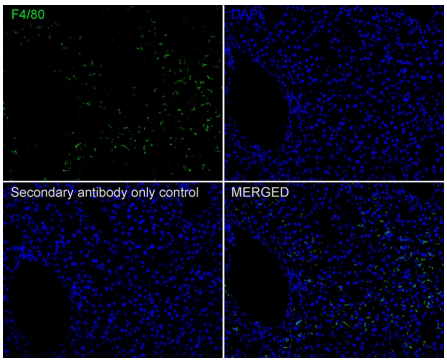

Fig6: Immunofluorescence analysis of paraffin-embedded mouse liver tissue labeling F4/80 with Rabbit anti-F4/80 antibody (HA721520) at 1/500 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721520, green) at 1/500 dilution overnight at 4 ℃, washed with PBS.

Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

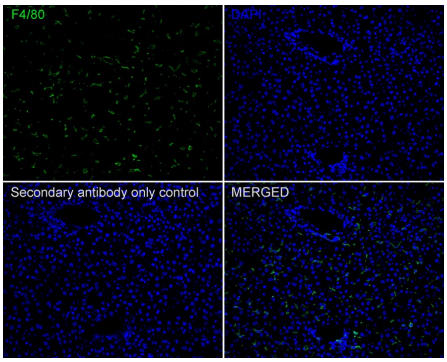

Fig7: Immunofluorescence analysis of paraffin-embedded rat liver tissue labeling F4/80 with Rabbit anti-F4/80 antibody (HA721520) at 1/500 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721520, green) at 1/500 dilution overnight at 4 ℃, washed with PBS.

Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

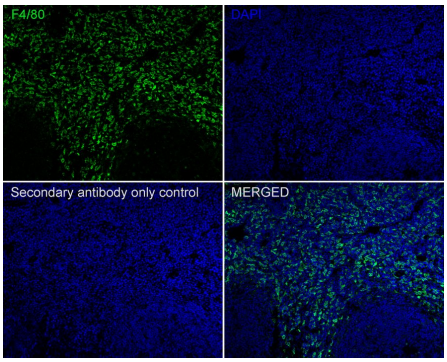

Fig8: Immunofluorescence analysis of frozen mouse spleen tissue with Rabbit anti-F4/80 antibody (HA721520) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721520, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

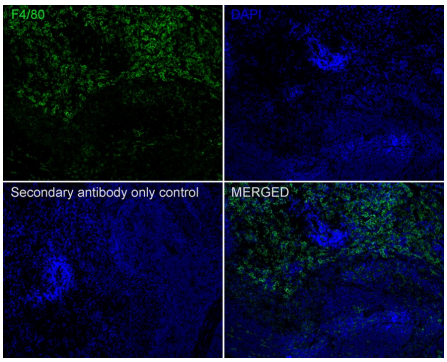

Fig9: Immunofluorescence analysis of frozen rat spleen tissue with Rabbit anti-F4/80 antibody (HA721520) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721520, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

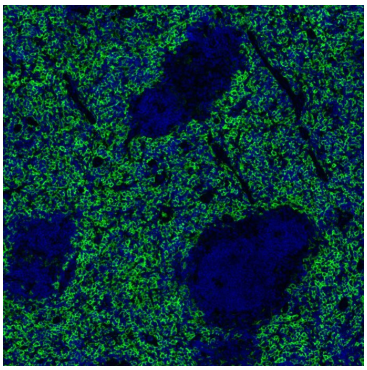

Fig10: mIHC analysis of mouse spleen tissue (Formalin/PFA-fixed paraffin-embedded sections) with Rabbit anti-F4/80 antibody (HA721520) at 1/500 dilution. The immunostaining was performed with the IRISKit® HyperView mTSA Kit (MH900206). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

Background References

1. Deng R et al. Periosteal CD68+ F4/80+ Macrophages Are Mechanosensitive for Cortical Bone Formation by Secretion and Activation of TGF-β1. Adv Sci (Weinh). 2022 Jan
2. Shin AE et al. F4/80+Ly6Chigh Macrophages Lead to Cell Plasticity and Cancer Initiation in Colitis. Gastroenterology. 2023 Apr