Anti-Ferritin Heavy Chain Antibody [SC0620] - BSA and Azide free

HA751971

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Zebrafish, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, IHC-Fr
Molecular Wt:	Predicted band size: 21 kDa
Clone number:	SC0620

Description: Ferritin is a universal intracellular protein that stores iron and releases it in a controlled fashion. The protein is produced by almost all living organisms, including archaea, bacteria, algae, higher plants, and animals. It is the primary intracellular iron-storage protein in both prokaryotes and eukaryotes, keeping iron in a soluble and non-toxic form. In humans, it acts as a buffer against iron deficiency and iron overload. Ferritin is found in most tissues as a cytosolic protein, but small amounts are secreted into the serum where it functions as an iron carrier. Plasma ferritin is also an indirect marker of the total amount of iron stored in the body; hence, serum ferritin is used as a diagnostic test for iron-deficiency anemia. Aggregated ferritin transforms into a toxic form of iron called hemosiderin. Ferritin is a globular protein complex consisting of 24 protein subunits forming a hollow nanocage with multiple metal–protein interactions. Ferritin that is not combined with iron is called apoferritin.

Immunogen: Synthetic peptide within Human FTH1 aa 58-99 / 183.

Positive control: 293T cell lysate, RAW264.7 cell lysate, Mouse liver tissue lysate, Mouse brain tissue lysate, Rat liver tissue lysate, Rat brain tissue lysate, SGC-7901, Jurkat, human spleen tissue, mouse liver tissue, rat liver tissue, rat spleen tissue.

Subcellular location: Cytoplasm.

Database links: SwissProt: P02794 Human | P09528 Mouse | P19132 Rat

Recommended Dilutions:

WB	1:5,000-1:20,000
IF-Cell	1:100
IF-Tissue	1:200
IHC-P	1:1,000
FC	1:1,000
IHC-Fr	1:500

Storage Buffer: PBS (pH7.4).

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

Purity: Protein A affinity purified.

Images

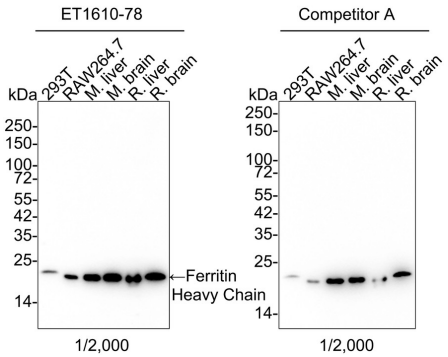

Fig1: Western blot analysis of Ferritin Heavy Chain on different lysates with Rabbit anti-Ferritin Heavy Chain antibody (HA751971) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution.

Lane 1: 293T cell lysate (30 µg/Lane)
Lane 2: RAW264.7 cell lysate (15 µg/Lane)
Lane 3: Mouse liver tissue lysate (15 µg/Lane)
Lane 4: Mouse brain tissue lysate (15 µg/Lane)
Lane 5: Rat liver tissue lysate (15 µg/Lane)
Lane 6: Rat brain tissue lysate (30 µg/Lane)

Predicted band size: 21 kDa
Observed band size: 21 kDa

Exposure time: 3 minutes 20 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 (HA751971) at 1/2,000 dilution and competitor's antibody at 1/2,000 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.

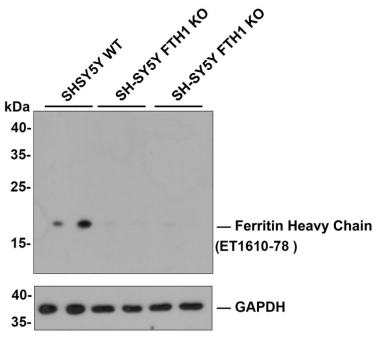

Fig2: All lanes: Western blot analysis of Ferritin Heavy Chain with anti-Ferritin Heavy Chain antibody (HA751971) at 1/5,000 dilution.
Lane 1/2: Wild-type SH-SY5Y whole cell lysate (10 µg).
Lane 3/4: Ferritin Heavy Chain fragment 1 knockout SH-SY5Y whole cell lysate (10 µg).
Lane 5/6: Ferritin Heavy Chain fragment 1 knockout SH-SY5Y whole cell lysate (10 µg).

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (HA751971, 1/5,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG-HRP Secondary Antibody (HA1001) at 1/200,000 dilution was used for 1 hour at room temperature.

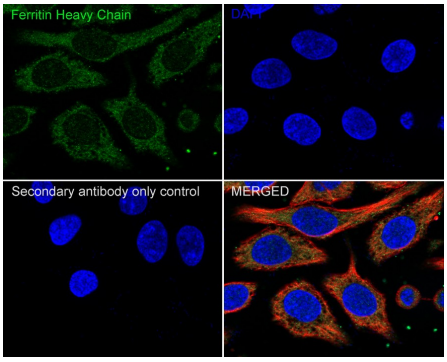

Fig3: Immunocytochemistry analysis of SGC-7901 cells labeling Ferritin Heavy Chain with Rabbit anti-Ferritin Heavy Chain antibody (HA751971) at 1/100 dilution.

Cells were fixed in 100% precooled methanol 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-Ferritin Heavy Chain antibody (HA751971) 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.

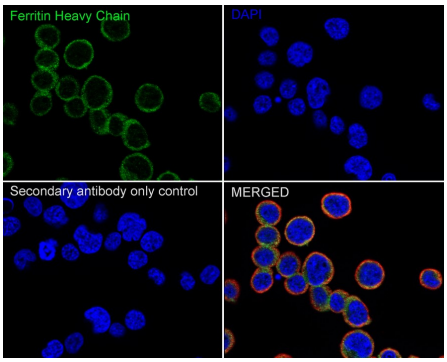

Fig4: Immunocytochemistry analysis of Jurkat cells labeling Ferritin Heavy Chain with Rabbit anti-Ferritin Heavy Chain antibody (HA751971) at 1/100 dilution.

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-Ferritin Heavy Chain antibody (HA751971) 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 (HA601187, 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.

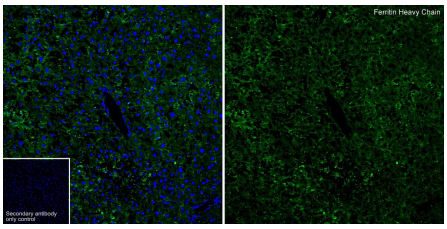

Fig5: Application: IHC-Fr

Species: Mouse

Site: liver

Sample: Frozen section

Antibody concentration: 1/500

Antigen retrieval: Not required

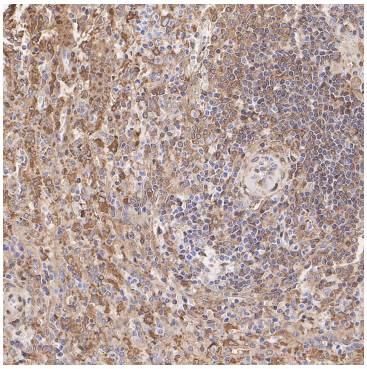

Fig6: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-Ferritin Heavy Chain antibody (HA751971) at 1/1,000 dilution.

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 (HA751971) 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.

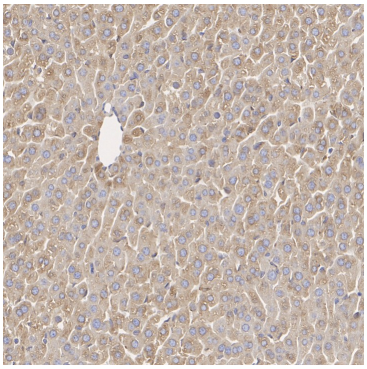

Fig7: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-Ferritin Heavy Chain antibody (HA751971) at 1/1,000 dilution.

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 (HA751971) 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.

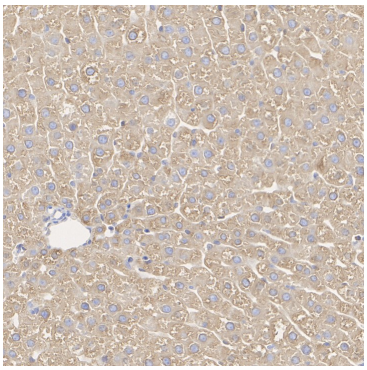

Fig8: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-Ferritin Heavy Chain antibody (HA751971) at 1/1,000 dilution.

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 (HA751971) 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.

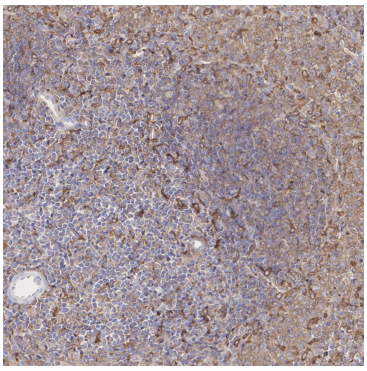

Fig9: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-Ferritin Heavy Chain antibody (HA751971) at 1/1,000 dilution.

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 (HA751971) 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.

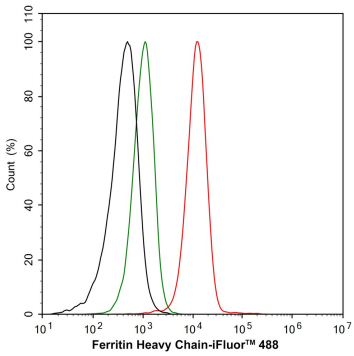

Fig10: Flow cytometric analysis of Jurkat cells labeling Ferritin Heavy Chain.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA751971, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ 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℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

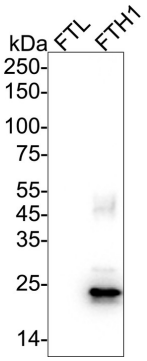

Fig11: Western blot analysis of Ferritin Heavy Chain on different lysates with Rabbit anti-Ferritin Heavy Chain antibody (HA751971) at 1/10,000 dilution.

Lane 1: FTL recombinant protein, 20ng/Lane
Lane 2: FTH1 recombinant protein, 20ng/Lane

Exposure time: 20 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 (HA751971) at 1/10,000 dilution was 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.

Background References

1. Ben-Othman R et al. Leishmania-mediated inhibition of iron export promotes parasite replication in macrophages. PLoS Pathog 10:e1003901 (2014).
2. Chin D et al. Curcumin may impair iron status when fed to mice for six months. Redox Biol 2:563-9 (2014).