abcam

ab215982

Anti-Ferritin heavy chain antibody [EPR3004Y] - Low endotoxin, Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR3004Y
Purification technique	Affinity purification Protein A
Specificity	We recommend following our protocol when testing ICC and recommend using lower dilution in samples having low expression level of Ferritin.
Concentration	0.978 - 0.988 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	We recommend following our protocol when testing ICC and recommend using lower dilution in samples having low expression level of Ferritin.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information FTH1

Function	Stores iron in a soluble, non-toxic, readily available form. Important for iron homeostasis. Has ferroxidase activity (PubMed:9003196). Iron is taken up in the ferrous form and deposited as ferric hydroxides after oxidation (PubMed:9003196). Also plays a role in delivery of iron to cells (By similarity). Mediates iron uptake in capsule cells of the developing kidney (By similarity). Delivery to lysosomes is mediated by the cargo receptor NCOA4 for autophagic degradation and release of iron (PubMed:24695223, PubMed:26436293).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab215982 is the carrier-free version of ab75973.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

What does low endotoxin mean?
Our low endotoxin, azide-free formats have low endotoxin level (1 EU/mg, determined by the TAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

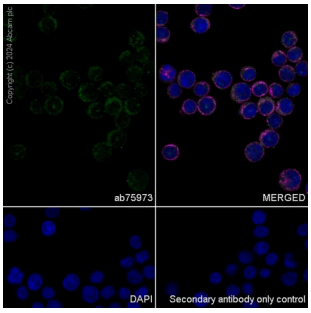
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

7 product images

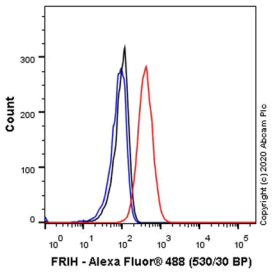


Immunocytochemistry/ Immunofluorescence - Anti-Ferritin heavy chain antibody [EPR3004Y] - Low endotoxin, Azide free (ab215982)

Immunofluorescence staining of 4% paraformaldehyde fixed, 0.1% Triton X-100 permeabilized Jurkat cells (human T cell leukemia T lymphocyte from peripheral blood) with ab75973 at a working dilution of 1/100. The secondary antibody, ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed, was used at a dilution of 1/1000. The counterstain, ab195889 anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used at a dilution of 1/200.

Confocal image showing positive staining in Jurkat cells (shown in green). The counterstain was observed in magenta. Nuclear DNA was labeled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

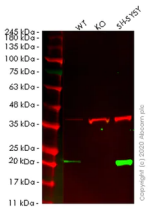
This data was developed using the same antibody clone in a different buffer formulation (ab75973).



Flow Cytometry (Intracellular) - Anti-Ferritin heavy chain antibody [EPR3004Y] - Low endotoxin, Azide free (ab215982)

Ferritin heavy chain Flow Cytometry (Intracellular) staining using rabbit Anti-Ferritin heavy chain antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized SH-SY5Y (Human neuroblastoma epithelial cell) cells labelling Ferritin heavy chain with ab75973 at 1/50 dilution (1µg) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab75973).



Western blot - Anti-Ferritin heavy chain antibody [EPR3004Y] - Low endotoxin, Azide free (ab215982)

Ferritin heavy chain Western blot staining using rabbit Anti-Ferritin heavy chain antibody

This data was developed using the same antibody clone in a different buffer formulation (ab75973).
Lanes 1-3: Merged signal (red and green). Green - ab75973 observed at 21 kDa. Red - loading control ab8245 observed at 36 kDa.

ab75973 Anti-Ferritin heavy chain antibody [EPR3004Y] was shown to specifically react with Ferritin heavy chain in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266581 (knockout cell lysate ab256924) was used. Wild-type and Ferritin knockout samples were subjected to SDS-PAGE. ab75973 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at room temperature for 2. 5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Ferritin heavy chain antibody [EPR3004Y] (ab75973) at 1/1000 dilution

Lane 1:
Wild-type HEK293T cell lysate at 20 µg

Lane 2:
Western blot - Human FTH1 knockout HEK-293T cell lysate (ab256924) at 20 µg

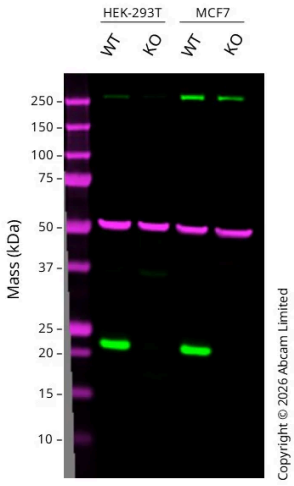
Lane 3:
SH-SY5Y cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 21 kDa

Observed band size: 21 kDa, 36 kDa



Western blot - Anti-Ferritin heavy chain antibody [EPR3004Y] - Low endotoxin, Azide free (ab215982)

Ferritin heavy chain Western blot staining using rabbit Anti-Ferritin heavy chain antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab75973).

Western blot: Anti-Ferritin heavy chain antibody [EPR3004Y] ab75973 staining at 1/1000 dilution, shown in green; Mouse anti alpha Tubulin ab7291 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 21 kDa in Wild-type HEK-293T cell lysates with no signal observed at this size in FTH1 knockout HEK-293T cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-Ferritin heavy chain antibody [EPR3004Y] (ab75973) at 1/1000 dilution

Lane 1:
Wild-type HEK-293T cell lysates at 20 µg

Lane 2:
Western blot - Human FTH1 knockout HEK-293T cell line (ab266581) at 20 µg

Lane 3:
Wild-type MCF7 cell lysates at 20 µg

Lane 4:
Western blot - Human FTH1 knockout MCF7 cell line (ab269493) at 20 µg

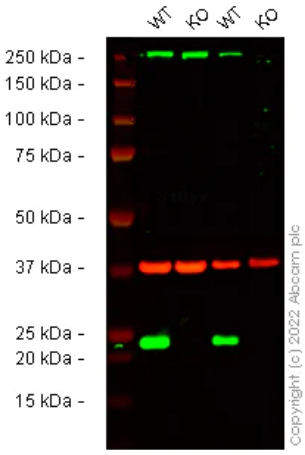
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 21 kDa

Observed band size: 21 kDa



Western blot - Anti-Ferritin heavy chain antibody [EPR3004Y] - Low endotoxin, Azide free (ab215982)

This data was developed using the same antibody clone in a different buffer formulation (ab75973). False colour image of Western blot: Anti-Ferritin heavy chain antibody [EPR3004Y] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab75973 was shown to bind specifically to Ferritin heavy chain. A band was observed at 24 kDa in wild-type MCF7 cell lysates with no signal observed at this size in FTH1 knockout cell line ab269493 (knockout cell lysate ab269655). To generate this image, wild-type and FTH1 knockout MCF7 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-Ferritin heavy chain antibody [EPR3004Y] (ab75973) at 1/1000 dilution

Lane 1:
Wild-type MCF7 cell lysate at 20 µg

Lane 2:
FTH1 knockout MCF7 cell lysate at 20 µg

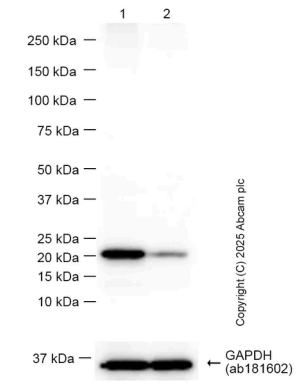
Lane 3:
Wild-type HEK-293T ab255553 at 20 µg

Lane 4:
FTH1 knockout HEK-293T ab260185 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 21 kDa

Observed band size: 24 kDa



Western blot - Anti-Ferritin heavy chain antibody [EPR3004Y] - Low endotoxin, Azide free (ab215982)

This data was developed using ab75973, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

All lanes:
Western blot - Anti-Ferritin heavy chain antibody [EPR3004Y] (ab75973) at 1/1000 dilution

Lane 1:
HeLa (Human cervix adenocarcinoma epithelial cell) + si -scramble whole cell lysate at 20 µg

Lane 2:
HeLa (Human cervix adenocarcinoma epithelial cell) + si - Ferritin Heavy Chain whole cell lysate at 20 µg

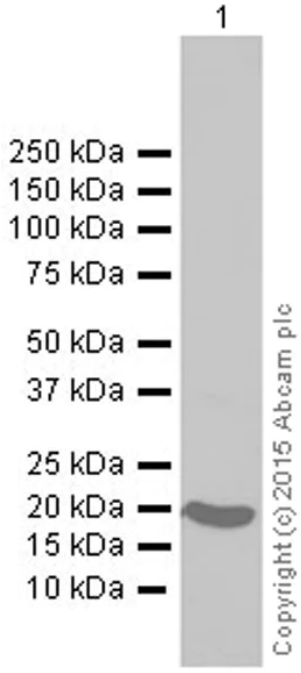
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 21 kDa

Observed band size: 21 kDa

Exposure time: 3s



Western blot - Anti-Ferritin heavy chain antibody [EPR3004Y] - Low endotoxin, Azide free (ab215982)

This data was developed using the same antibody clone in a different buffer formulation (ab75973). Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-Ferritin heavy chain antibody [EPR3004Y] (ab75973) at 1/10000 dilution

All lanes:
Raw264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 21 kDa

Observed band size: 21 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.