abcam

Product datasheet

Anti-Ferritin antibody [EPR3004Y] ab75973



יעלטעבע RabMAb

★★★★ 7 Abreviews 86 References 画像数8

製品の概要

製品名 Anti-Ferritin antibody [EPR3004Y]

製品の詳細 Rabbit monoclonal [EPR3004Y] to Ferritin

由来種 Rabbit

特異性 We recommend following our protocol when testing ICC and recommend using lower dilution in

samples having low expression level of Ferritin.

アプリケーション 適用あり: ICC/IF, Flow Cyt (Intra), WB

適用なし: IHC-P

種交差性 交差種: Mouse, Rat, Human

免疫原 Synthetic peptide within Human Ferritin aa 50-150. The exact sequence is proprietary.

Database link: P02794

ポジティブ・コントロール WB: HEK293T, Jurkat, SH-SY-5Y, C6 and Raw264.7 cell lysates; Human and rat brain lysates

ICC/IF: Jurkat cells Flow Cyt (intra): SH-SY5Y cells

特記事項 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 production

For more information see here.

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

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA

精製度 Protein A purified

ポリÆノ モノクローナル **ウローン名** EPR3004Y

アイソタイプ lgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab75973の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
ICC/IF		1/100. We recommend following our protocol when testing ICC and recommend using lower dilution in samples having low expression level of Ferritin.
Flow Cyt (Intra)		1/50.
WB	★★★★★ (6)	1/1000 - 1/2000. Detects a band of approximately 19, 21 kDa (predicted molecular weight: 21,19 kDa).

追加情報 Is unsuitable for IHC-P.

ターゲット情報

機能
Stores iron in a soluble, non-toxic, readily available form. Important for iron homeostasis. Has ferroxidase activity. Iron is taken up in the ferrous form and deposited as ferric hydroxides after oxidation. Also plays a role in delivery of iron to cells. Mediates iron uptake in capsule cells of the developing kidney.

配列類似性 Belongs to the ferritin family.

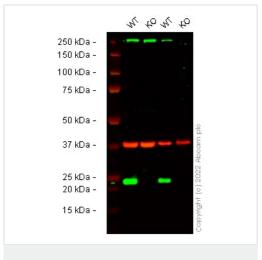
Contains 1 ferritin-like diiron domain.

製品の状態
Subunit structure: Oligomer of 24 subunits. There are two types of subunits: L (light) chain and H

(heavy) chain. The major chain can be light or heavy, depending on the species and tissue type. In the human liver, the heavy chain is predominant. The functional molecule forms a roughly spherical shell with a diameter of 12 nm and contains a central cavity into which the insoluble mineral iron

core is deposited.

画像



Western blot - Anti-Ferritin antibody [EPR3004Y] (ab75973)

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

Lane 1: Wild-type MCF7 cell lysate

Lane 2: FTH1 knockout MCF7 cell lysate

Lane 3: Wild-type HEK-293T ab255553 cell lysate

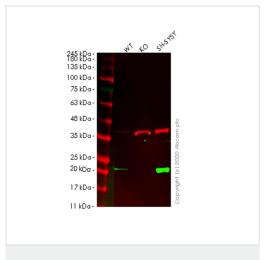
Lane 4: FTH1 knockout HEK-293T ab260185 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 21,19 kDa **Observed band size:** 24 kDa

False colour image of Western blot: Anti-Ferritin 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. 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.



Western blot - Anti-Ferritin antibody [EPR3004Y] (ab75973)

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

Lane 1: Wild-type HEK293T cell lysate

Lane 2: FTH1 knockout HEK293T cell lysate

Lane 3: SH-SY5Y cell lysate

Lysates/proteins at 20 µg per lane.

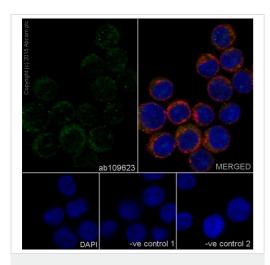
Secondary

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

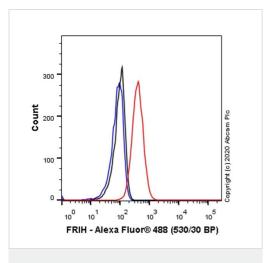
Predicted band size: 21,19 kDa **Observed band size:** 21 kDa

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 antibody [EPR3004Y] was shown to specifically react with Ferritin in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266581 (knockout cell lysate ab266924) 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 lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216773) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Ferritin antibody [EPR3004Y] (ab75973)



Flow Cytometry (Intracellular) - Anti-Ferritin antibody [EPR3004Y] (ab75973)

Immunofluorescence staining of Jurkat cells with purified ab75973 at a working dilution of 1/100, counter-stained with DAPI. The secondary antibody was Alexa Fluor[®] 488 goat anti-rabbit (ab150077), used at a dilution of 1/1000. ab7291, a mouse antitubulin antibody (1/1000), was used to stain tubulin along with ab150120 (Alexa Fluor[®] 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 100% methanol and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab75973 was used at a dilution of 1/500 followed by an Alexa Fluor[®] 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500. For negative control 2, ab7291 (mouse antitubulin) was used at a dilution of 1/500 followed by an Alexa Fluor[®] 488 goat anti-rabbit antibody (ab150077) at a dilution of 1/400.

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized SH-SY5Y (Human neuroblastoma epithelial cell) cells labelling Ferritin 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.



Western blot - Anti-Ferritin antibody [EPR3004Y] (ab75973)

All lanes : Anti-Ferritin antibody [EPR3004Y] (ab75973) at 1/2000 dilution

Lane 1: Jurkat (Human T cell leukemia T lymphocyte) whole cell lysates

Lane 2: HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates

Lane 3: C6 (Rat glial tumor glial cell) whole cell lysates

Lysates/proteins at 20 µg per lane.

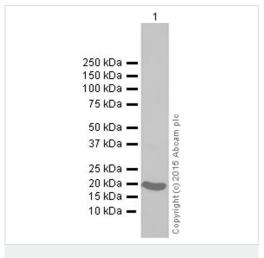
Secondary

All lanes : Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/20000 dilution

Predicted band size: 21,19 kDa

Observed MW: 21 kDA

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



Western blot - Anti-Ferritin antibody [EPR3004Y] (ab75973)

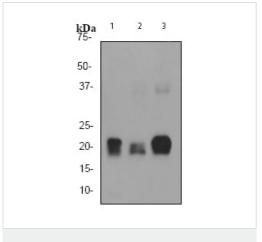
Anti-Ferritin antibody [EPR3004Y] (ab75973) at 1/10000 dilution (purified) + Raw264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate at 10 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 21,19 kDa **Observed band size:** 21 kDa

Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST



Western blot - Anti-Ferritin antibody [EPR3004Y] (ab75973)

All lanes : Anti-Ferritin antibody [EPR3004Y] (ab75973) at 1/2000 dilution (Unpurified)

Lane 1 : Jurkat (Human T cell leukemia cell line from peripheral blood) cell lysate

Lane 2: SH-SY5Y (Human neuroblastoma cell line from bone marrow) cell lysate

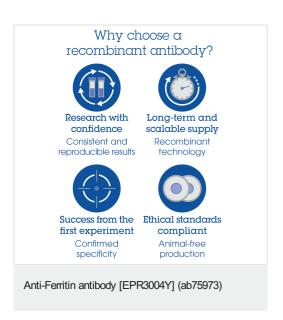
Lane 3: Human brain lysates

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 21,19 kDa **Observed band size:** 19,21 kDa



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