abcam

Product datasheet

Anti-Transferrin Receptor antibody [EPR20584] ab214039



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Overview

Product name Anti-Transferrin Receptor antibody [EPR20584]

Description Rabbit monoclonal [EPR20584] to Transferrin Receptor

Host species Rabbit

Tested applications Suitable for: IHC-P, ICC/IF, IP, WB

Species reactivity Reacts with: Mouse, Human

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human fetal liver and placenta lysates; Mouse spleen, testis and placenta lysates; K562, TF-

1, Jurkat and RAW 264.7 whole cell lysates; HeLa, HUVEC, 293T, mouse lung and mouse brain whole cell lysates. IHC-P: Human placenta, cerebrum, esophagus and esophageal cancer tissues; Mouse kidney tissue. ICC/IF: K562 and RAW 264.7 cells. IP: K562 whole cell lysate.

General notesThis 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**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 0.05% BSA, 40% Glycerol, PBS

Purity Protein A purified

1

ClonalityMonoclonalClone numberEPR20584

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab214039 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P	****(1)	1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/100.
IP		1/30.
WB		1/1000. Detects a band of approximately 90 kDa (predicted molecular weight: 85 kDa).

Target

Function

Cellular uptake of iron occurs via receptor-mediated endocytosis of ligand-occupied transferrin receptor into specialized endosomes. Endosomal acidification leads to iron release. The apotransferrin-receptor complex is then recycled to the cell surface with a return to neutral pH and the concomitant loss of affinity of apotransferrin for its receptor. Transferrin receptor is necessary for development of erythrocytes and the nervous system (By similarity). A second ligand, the heditary hemochromatosis protein HFE, competes for binding with transferrin for an overlapping C-terminal binding site. Positively regulates T and B cell proliferation through iron uptake (PubMed:26642240).

(Microbial infection) Acts as a receptor for new-world arenaviruses: Guanarito, Junin and Machupo virus.

Involvement in disease Immunodeficiency 46

Sequence similarities Belongs to the peptidase M28 family. M28B subfamily.

Contains 1 PA (protease associated) domain.

Post-translational modifications

N- and O-glycosylated, phosphorylated and palmitoylated. The serum form is only glycosylated.

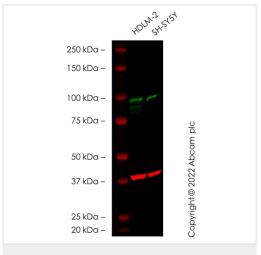
Proteolytically cleaved on Arg-100 to produce the soluble serum form (sTfR).

Palmitoylated on both Cys-62 and Cys-67. Cys-62 seems to be the major site of palmitoylation.

Cellular localization Secreted and Cell membrane. Melanosome. Identified by mass spectrometry in melanosome

fractions from stage I to stage IV.

Images



Western blot - Anti-Transferrin Receptor antibody [EPR20584] (ab214039)

All lanes : Anti-Transferrin Receptor antibody [EPR20584] (ab214039) at 1/1000 dilution

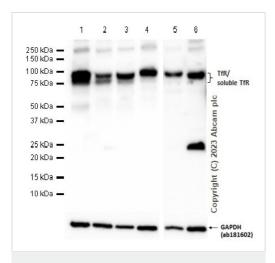
Lane 1 : HDLM-2 cell lysate
Lane 2 : SH-SY5Y cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 85 kDa **Observed band size:** 90 kDa

False colour image of Western blot: Anti-Transferrin Receptor antibody [EPR20584] 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, ab214039 was shown to bind specifically to Transferrin Receptor. 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 lgG H&L 800CW and Goat anti-Mouse lgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-Transferrin Receptor antibody [EPR20584] (ab214039)

All lanes : Anti-Transferrin Receptor antibody [EPR20584] (ab214039) at 1/1000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 2 : HUVEC (Human umbilical vein endothelial cell) whole cell lysate

Lane 3: 293T (Human embryonic kidney epithelial cell) whole cell lysate

Lane 4: Raw 264.7 (Mouse Abelson murine leukemia virusinduced tumor macrophage) whole cell lysate

Lane 5 : Mouse lung tissue lysate

Lane 6: Mouse brain tissue

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 85 kDa **Observed band size:** 75,85 kDa

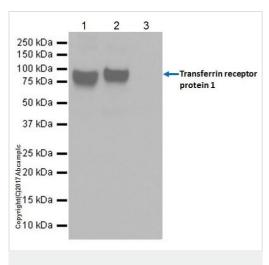
Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM/TBST

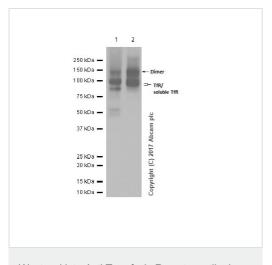
Exposure Time: Lane 1-4: 3 seconds, Lane 5-6: 20 seconds.

Anti-GAPDH antibody [EPR16891] - Loading Control (<u>ab181602</u>) used.

Soluble transferrin receptor (sTfR) is cleaved from whole TfR at Arg 100. (PMID: 9575164, PMID: 23390091).



Immunoprecipitation - Anti-Transferrin Receptor antibody [EPR20584] (ab214039)



Western blot - Anti-Transferrin Receptor antibody [EPR20584] (ab214039)

Transferrin Receptor was immunoprecipitated from 0.35 mg of K562 (human chronic myelogenous leukemia cell line from bone marrow) lysate with ab214039 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab214039 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10,000 dilution

Lane 1: K562 whole cell lysate 10 µg (Input).

Lane 2: ab214039 IP in K562 whole cell lysate.

Lane 3: Rabbit monoclonal $\lg G$ ($\underline{ab172730}$) instead of ab214039 in K562 whole cell lysate.

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

Exposure time: 1 second.

All lanes : Anti-Transferrin Receptor antibody [EPR20584] (ab214039) at 1/1000 dilution

Lane 1 : Daudi (Human Burkitt's lymphoma lymphoblast) whole cell lysate

Lane 2: K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 85 kDa

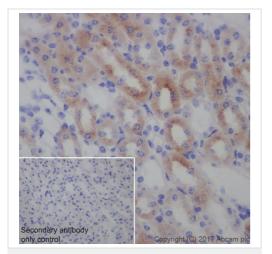
Observed band size: 150,75,85 kDa

Exposure time: 3 seconds

Soluble transferrin receptor (sTfR) is cleaved from whole TfR at Arg 100. (PMID: 9575164, PMID: 23390091)

TfR is found as a dimer linked by disulfide bonds in cell, so sometimes we could see dimer in a few kinds of lysates. (PMID: 2507316, PMID: 3582362). This dimer was also be detected by **ab108985**.

Blocking buffer: 5% NFDM/TBST.

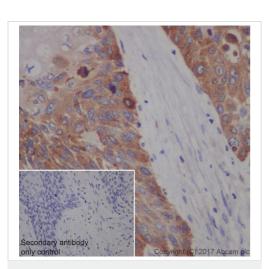


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Transferrin Receptor antibody [EPR20584] (ab214039)

Immunohistochemical analysis of paraffin-embedded mouse kidney tissue labeling Transferrin Receptor with ab214039 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on renal tubules of mouse kidney (PMID: 12538733). Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

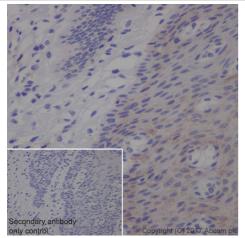


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Transferrin Receptor antibody [EPR20584] (ab214039)

Immunohistochemical analysis of paraffin-embedded human esophageal cancer tissue labeling Transferrin Receptor with ab214039 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on human esophageal cancer. The staining intensity of human esophageal cancer tissue was stronger than the human paracarcinoma esophagus tissue. Both paracarcinoma and human esophageal cancer tissues have been taken from the same patient (PMID: 24435655). Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



embedded sections) - Anti-Transferrin Receptor antibody [EPR20584] (ab214039)

Immunohistochemistry (Formalin/PFA-fixed paraffin-

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Transferrin Receptor antibody [EPR20584] (ab214039)

Immunohistochemical analysis of paraffin-embedded human esophagus tissue labeling Transferrin Receptor with ab214039 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Weakly cytoplasmic staining on human paracarcinoma esophagus. The staining intensity of human paracarcinoma esophagus was weaker than the human esophageal cancer tissue. Both paracarcinoma and human esophageal cancer tissues have been taken from the same patient (PMID: 24435655). Counter stained with Hematoxylin.

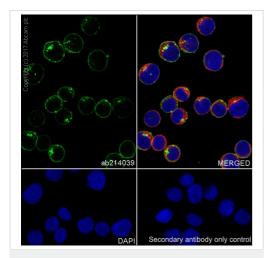
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Immunohistochemical analysis of paraffin-embedded human cerebrum tissue labeling Transferrin Receptor with ab214039 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Positive staining on capillaries of human cerebrum (PMID: 6095085). Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

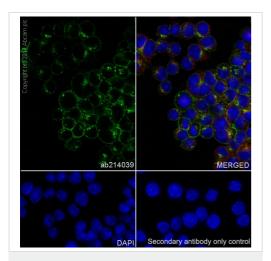


Immunocytochemistry/ Immunofluorescence - Anti-Transferrin Receptor antibody [EPR20584] (ab214039)

Immunofluorescent analysis of 100% methanol-fixed K562 (human chronic myelogenous leukemia cell line from bone marrow) cells labeling Transferrin Receptor with ab214039 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing positive staining on K562 cells.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) (ab195889) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution.

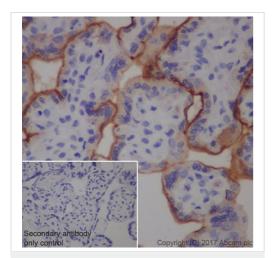


Immunocytochemistry/ Immunofluorescence - Anti-Transferrin Receptor antibody [EPR20584] (ab214039)

Immunofluorescent analysis of 100% methanol-fixed RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) cells labeling Transferrin Receptor with ab214039 at 1/100 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing positive staining on RAW 264.7 cells.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) (ab195889) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution.



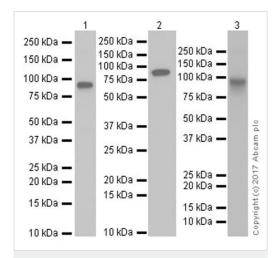
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Transferrin Receptor antibody [EPR20584] (ab214039)

Immunohistochemical analysis of paraffin-embedded human placenta tissue labeling Transferrin Receptor with ab214039 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Positive staining on human placenta (PMID: 27483296). Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-Transferrin Receptor antibody [EPR20584] (ab214039)

Lanes 1-2: Anti-Transferrin Receptor antibody [EPR20584] (ab214039) at 1/2000 dilution

Lane 3: Anti-Transferrin Receptor antibody [EPR20584] (ab214039) at 1/1000 dilution

Lane 1 : K562 (human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate at 10 μ g

Lane 2 : Jurkat (human T cell leukemia cell line from peripheral blood) whole cell lysate at 10 µg

Lane 3 : TF-1 (human bone marrow erythroleukemia cell line) whole cell lysate at 20 μg

Secondary

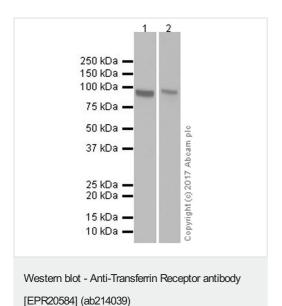
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 85 kDa
Observed band size: 90 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 2 seconds; Lane 2: 1 minute; Lane 3: 1

second.



All lanes: Anti-Transferrin Receptor antibody [EPR20584] (ab214039) at 1/1000 dilution

Lane 1: Human fetal liver lysate Lane 2: Human placenta lysate

Lysates/proteins at 20 µg per lane.

Secondary

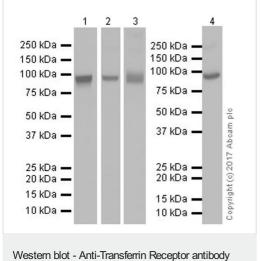
All lanes: VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/4000 dilution

Predicted band size: 85 kDa

Observed band size: 90 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 3 seconds; Lane 2: 15 seconds.



[EPR20584] (ab214039)

All lanes: Anti-Transferrin Receptor antibody [EPR20584] (ab214039) at 1/1000 dilution

Lane 1: Mouse spleen lysate at 20 µg

Lane 2: Mouse testis lysate at 20 µg

Lane 3: Mouse placenta lysate at 20 µg

Lane 4: RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate at 10 μg

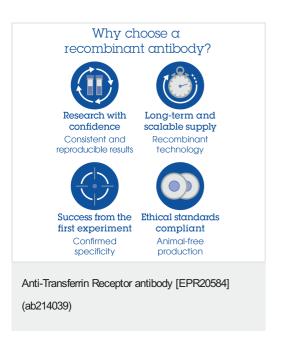
Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 85 kDa Observed band size: 90 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1/4: 3 seconds; Lane 2: 30 seconds; Lane 3:



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