abcam

Product datasheet

Anti-CFHR3 antibody [EPR25759-72] ab302792

Recombinant RabMAb

6 Images

.

Overview		
Product name	Anti-CFHR3 antibody [EPR25759-72]	
Description	Rabbit monoclonal [EPR25759-72] to Complement factor H-related protein 3	
Host species	Rabbit	
Tested applications	Suitable for: WB, IP, ICC/IF Unsuitable for: Flow Cyt (Intra) or IHC-P	
Species reactivity	Reacts with: Human Does not react with: Mouse, Rat	
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.	
Positive control	WB: Whole cell lysates: Daudi (human Burkitt's lymphoma lymphoblast), Raji (human Burkitt's lymphoma B lymphocyte), Low expression: HepG2 (human hepatocellular carcinoma epithelial cell), untreated human plasma, hman plasma treated with Protein Deglycosylation Mix II, His- tagged human CFHR3 recombinant protein. ICC/IF: Raji (human Burkitt's lymphoma B lymphocyte). IP: Raji (human Burkitt's lymphoma B lymphocyte).	
General notes	ab302792 does not react in: WB with mouse and rat samples; ICC/IF and IP with mouse tissues; intracellular flow cytometry with human and mouse specimens.	
	 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 <u>see here</u>. Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb[®] patents</u>. 	

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: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR25759-72
lsotype	lgG

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab302792 in the following tested applications.

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

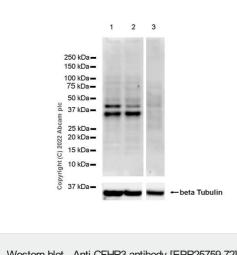
Application	Abreviews	Notes
WB		1/1000. Predicted molecular weight: 37 kDa. Observed Mass of target (kDa) 40, 35,50, 37, 35,45-50
IP		1/30.
ICC/IF		1/50.

Application notes

Is unsuitable for Flow Cyt (Intra) or IHC-P.

Target	
Function	Might be involved in complement regulation.
Tissue specificity	Expressed by the liver and secreted in plasma.
Sequence similarities	Contains 5 Sushi (CCP/SCR) domains.
Cellular localization	Secreted.

Images



Western blot - Anti-CFHR3 antibody [EPR25759-72] (AB302792) All lanes : Anti-CFHR3 antibody [EPR25759-72] (ab302792) at 1/1000 dilution

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

Lane 2 : Raji (human Burkitt's lymphoma B lymphocyte), whole cell lysate

Lane 3 : HepG2 (human hepatocellular carcinoma epithelial cell), 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

Developed using the ECL technique.

Predicted band size: 37 kDa Observed band size: 40, 35 kDa

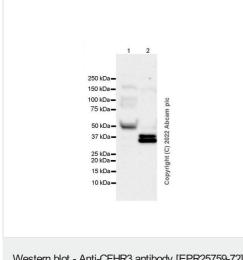
Exposure time: 37 seconds

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

The doublet bands represent two isoforms of CFHR3 (PMID: 8428964).

Low expression: HepG2 (Human Protein Atlas)

This blot was developed using a high sensitivity ECL substrate.



Western blot - Anti-CFHR3 antibody [EPR25759-72] (AB302792) All lanes : Anti-CFHR3 antibody [EPR25759-72] (ab302792) at 1/1000 dilution

Lane 1 : Untreated human plasma

Lane 2: Human plasma treated with Protein Deglycosylation Mix II

Lysates/proteins at 15 µg per lane.

Secondary

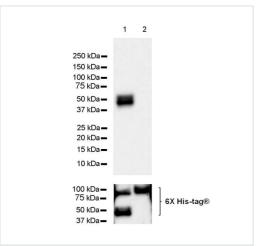
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 37 kDa Observed band size: 50, 37, 35 kDa

Exposure time: 103 seconds

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

CFHR3 is a glycoprotein of approximately 50 kDa and detected as 37/35 kDa bands after Protein Deglycosylation Mix II treatment. (PMID: 23830046)



Western blot - Anti-CFHR3 antibody [EPR25759-72] (AB302792) All lanes : Anti-CFHR3 antibody [EPR25759-72] (ab302792) at 1/1000 dilution

Lane 1 : His-tagged human CFHR3 recombinant protein 10 ng Lane 2 : His-tagged human CFHR4 recombinant protein 10 ng

Secondary

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

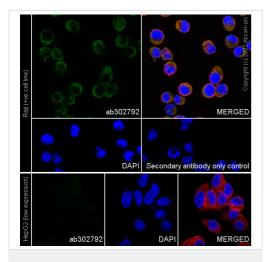
Predicted band size: 37 kDa Observed band size: 45-50 kDa

Exposure time: 10 seconds

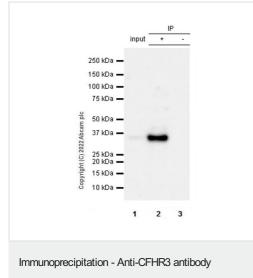
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

This antibody does not cross-react with human CFHR4.

Both recombinant proteins were made in-house and expressed from a mammalian expression system.



Immunocytochemistry/ Immunofluorescence - Anti-CFHR3 antibody [EPR25759-72] (AB302792)



[EPR25759-72] (AB302792)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized Raji (human Burkitt's lymphoma B lymphocyte) cells labeling CFHR3 with ab302792 at 1/50 dilution (10.74 µg/ml), followed by <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) preadsorbed antibody at 1/1000 dilution (2 µg/ml) (Green). Confocal image showing cytoplasmic staining in Raji cell line and weak staining in HepG2 cell line. Low expression is observed in HepG2. <u>ab195889</u> Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 dilution (2.5 µg/ml) (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) preadsorbed at 1/1000 dilution (2 µg/ml).

CFHR3 was immunoprecipitated from 0.35 mg Raji (human Burkitt's lymphoma B lymphocyte), whole cell lysate with ab302792 at 1/30 dilution (2 µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab302792 at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP)(**ab131366**) was used at 1/5000 dilution.

Lane 1: Raji (human Burkitt's lymphoma B lymphocyte), whole cell lysate 10 µg (Inset)

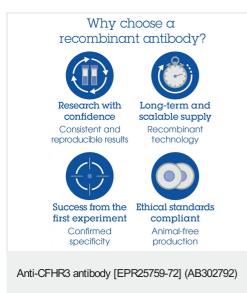
Lane 2: ab302792 IP in Raji whole cell lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab302792 in Raji whole cell lysate

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

Exposure time: 10 seconds

Obeserved M_W (kDa): 35.



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