abcam

Product datasheet

Anti-C5 / C5b antibody [EPR23940-3] ab275931

Recombinant RabMAb

1 References 6 Images

Overview

Product name Anti-C5 / C5b antibody [EPR23940-3]

Description Rabbit monoclonal [EPR23940-3] to C5 / C5b

Host species Rabbit

Tested applications Suitable for: WB, IHC-P

Unsuitable for: Flow Cyt,ICC/IF or IP

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: Human plasma and liver tissue lysate; Mouse plasma; Rat plasma. IHC-P: Human liver and

breast tissue.

General notes 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**.

Properties

Form Liquid

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

term. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.01% Sodium azide

Constituents: 59.94% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR23940-3

Isotype lgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab275931 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. Detects a band of approximately 70, 190 kDa (predicted molecular weight: 188 kDa).
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Application notes

Is unsuitable for Flow Cyt,ICC/IF or IP.

Target

Relevance

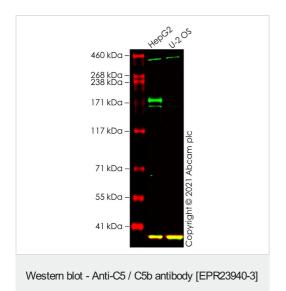
C5 is synthesised in the liver as a single polypeptide chain. Before secretion the molecule is glycosylated and secreted into plasma as a 190kDa glycoprotein consisting of a disulphide linked alpha-chain (115kDa) and beta-chain (75kDa). C5 precursor is first processed by the removal of 4 basic residues, forming two chains, beta and alpha, linked by a disulfide bond. C5 convertase activates C5 by cleaving the alpha chain, releasing C5a anaphylatoxin and generating C5b (beta chain + alpha' chain).

Cellular localization

Secreted

Images

(ab275931)



All lanes : Anti-C5 / C5b antibody [EPR23940-3] (ab275931) at 1/1000 dilution

Lane 1 : HepG2 cell lysate
Lane 2 : U-2 OS cell lysate

Lysates/proteins at 20 µg per lane.

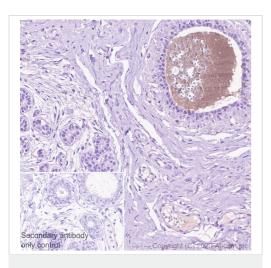
Performed under reducing conditions.

Predicted band size: 188 kDa

Observed band size: 165-180 kDa

False colour image of Western blot: Anti-C5 / C5b antibody [EPR23940-3] 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, ab275931 was

shown to bind specifically to C5 / C5b. 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 (IRDye[®] 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye[®] 680RD) preabsorbed (ab216776) at 1/20000 dilution.

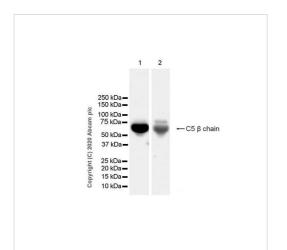


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-C5 / C5b antibody
[EPR23940-3] (ab275931)

Immunohistochemical analysis of paraffin-embedded human breast tissue labeling C5/C5b with ab275931 at 1/4000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on plasma of human breast. The section was incubated with ab275931 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Western blot - Anti-C5 / C5b antibody [EPR23940-3] (ab275931)

All lanes : Anti-C5 / C5b antibody [EPR23940-3] (ab275931) at 1/1000 dilution

Lane 1 : Mouse plasma
Lane 2 : Rat plasma

Lysates/proteins at 20 µg per lane.

Secondary

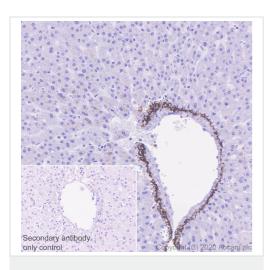
All lanes : Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 188 kDa **Observed band size:** 70 kDa

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

The molecular weight observed is consistent with what has been described in the literature (PMID: 23401592, 25387724).

Exposure time: 48 seconds.

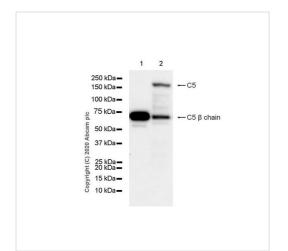


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-C5 / C5b antibody
[EPR23940-3] (ab275931)

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling C5/C5b with ab275931 at 1/4000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human liver. The section was incubated with ab275931 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Western blot - Anti-C5 / C5b antibody [EPR23940-3] (ab275931)

All lanes : Anti-C5 / C5b antibody [EPR23940-3] (ab275931) at 1/1000 dilution

Lane 1: Human plasma at 10 µg

Lane 2: Human liver tissue lysate at 40 µg

Secondary

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

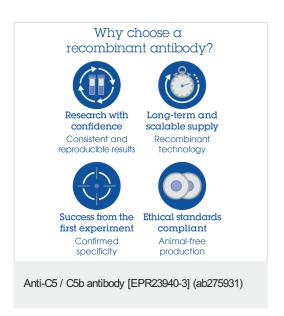
Predicted band size: 188 kDa **Observed band size:** 190,70 kDa

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

Samples are non-boiled as boiling may cause protein aggregates.

The molecular weight observed is consistent with what has been described in the literature (PMID: 24071736, 23401592, 25387724)

Exposure time: 59 seconds.



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