# abcam

# Product datasheet

# Anti-Caspase-6/CASP-6 antibody [EPR18043] ab185645

Recombinant RabMAb

# 1 Abreviews 12 References 9 Images

Overview

**Product name** Anti-Caspase-6/CASP-6 antibody [EPR18043]

**Description**Rabbit monoclonal [EPR18043] to Caspase-6/CASP-6

Host species Rabbit

Tested applications
Suitable for: WB, IHC-P, IP, ICC/IF
Species reactivity
Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Jurkat, NIH/3T3, C6 and PC-12 whole cell lysates. IHC-P: Human colon, mouse brain and rat

brain tissues. ICC/IF: Jurkat cells. IP: NIH/3T3 whole cell lysate treated with 1uM staurosporine for

4 hours.

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  $\underline{\text{see here}}$ .

Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **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: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EPR18043

1

**Isotype** IgG

#### **Applications**

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab185645 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 33, 11 kDa (predicted molecular weight: 33 kDa).                                   |
| IHC-P       |           | 1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| IP          |           | 1/50.                                                                                                                      |
| ICC/IF      |           | 1/500.                                                                                                                     |

#### **Target**

Function Involved in the activation cascade of caspases responsible for apoptosis execution. Cleaves

poly(ADP-ribose) polymerase in vitro, as well as lamins. Overexpression promotes programmed

cell death.

**Sequence similarities** Belongs to the peptidase C14A family.

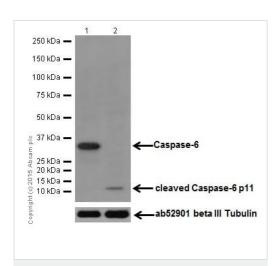
Post-translational

modifications

Cleavages by caspase-3, caspase-8 or -10 generate the two active subunits.

Cellular localization Cytoplasm.

#### **Images**



Western blot - Anti-Caspase-6/CASP-6 antibody [EPR18043] (ab185645)

**All lanes :** Anti-Caspase-6/CASP-6 antibody [EPR18043] (ab185645) at 1/1000 dilution

**Lane 1 :** Untreated Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate

Lane 2: Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate treated with 1uM staurosporine for 4 hours

Lysates/proteins at 10 µg per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit lgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 33 kDa Observed band size: 11,33 kDa

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.

250 kDa • 150 kDa -100 kDa 🕳 75 kDa 🕳 50 kDa — 37 kDa 🕳 -Caspase-6 25 kDa — 20 kDa -25 kDa — 15 kDa — 10 kDa — - cleaved Caspase-6 p11 -ab52901 beta III Tubulin

Western blot - Anti-Caspase-6/CASP-6 antibody [EPR18043] (ab185645)

All lanes: Anti-Caspase-6/CASP-6 antibody [EPR18043] (ab185645) at 1/1000 dilution

Lane 1: Untreated NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate

Lane 2: NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate treated with 1uM staurosporine for 4 hours

Lysates/proteins at 10 µg per lane.

### Secondary

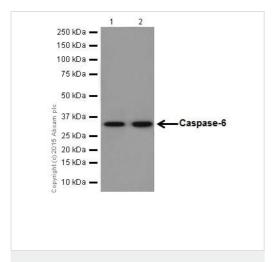
All lanes: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 33 kDa Observed band size: 11,33 kDa

Exposure time: 1 minute

The predicted MW is 32kDa for mouse and rat full-length procaspase-6.

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Caspase-6/CASP-6 antibody [EPR18043] (ab185645)

**All lanes :** Anti-Caspase-6/CASP-6 antibody [EPR18043] (ab185645) at 1/1000 dilution

Lane 1: C6 (Rat glial tumor cells) whole cell lysate

Lane 2 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell

lysate

Lysates/proteins at 10 µg per lane.

# Secondary

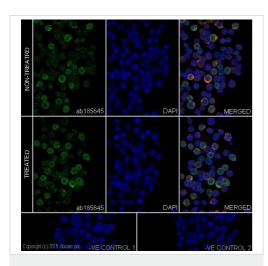
**All lanes :** Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 33 kDa **Observed band size:** 33 kDa

Exposure time: 1 minute

The predicted MW is 32kDa for mouse and rat full-length procaspase-6.

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-Caspase-6/CASP-6 antibody [EPR18043] (ab185645)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat (Human T cell leukemia cells from peripheral blood) cells labeling Caspase-6/CASP-6 with ab185645 at 1/500 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green).

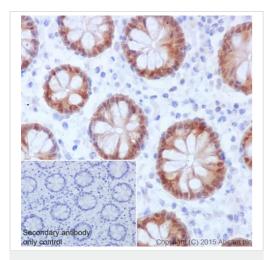
Confocal image showing both nuclear and cytoplasmic staining on Jurkat cells. The staining remained similar after treatment with staurosporine (1uM, 4 hours) as the antibody interacts with the subunit p11.

The nuclear counter stain is DAPI (blue).

Tubulin is detected with <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution and <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:

-ve control 1: ab185645 at 1/500 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Caspase-6/CASP-6 antibody [EPR18043] (ab185645)

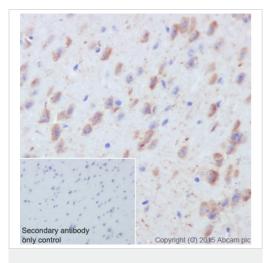
Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling Caspase-6/CASP-6 with ab185645 at 1/1000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution.

Cytoplasmic and nuclear staining on epithelial cells of human colon is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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-Caspase-6/CASP-6 antibody [EPR18043] (ab185645)

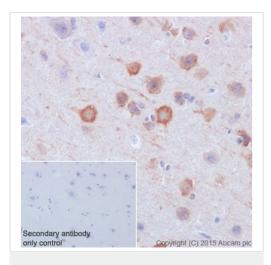
Immunohistochemical analysis of paraffin-embedded Mouse brain tissue labeling Caspase-6/CASP-6 with ab185645 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution.

Cytoplasmic staining on neurons of mouse brain is observed.

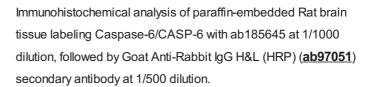
Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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-Caspase-6/CASP-6 antibody [EPR18043] (ab185645)

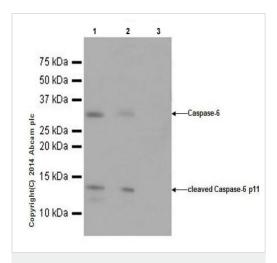


Cytoplasmic staining on neurons of rat brain is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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



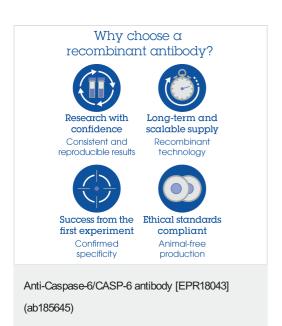
Immunoprecipitation - Anti-Caspase-6/CASP-6 antibody [EPR18043] (ab185645)

Caspase-6/CASP-6 was immunoprecipitated from 1mg of NIH/3T3 (Mouse embyro fibroblast cells) treated with 1uM staurosporine for 4 hours whole cell lysate with ab185645 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab185645 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1/1500 dilution.

Lane 1: NIH/3T3 treated with 1uM staurosporine for 4 hours whole cell lysate 10  $\mu$ g (Input). Lane 2: ab185645 IP in NIH/3T3 treated with 1uM staurosporine for 4 hours whole cell lysate. Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab185645 in NIH/3T3 treated with 1uM staurosporine for 4 hours whole cell lysate.

The cleaved Caspase-6/CASP-6 appears slightly larger than 11kDa. This fragment contains subunit p11 plus the internal propeptide.

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



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