# abcam

## Product datasheet

## Anti-CD3 epsilon antibody ab5690

#### Overview

Product name Anti-CD3 epsilon antibody

**Description** Rabbit polyclonal to CD3 epsilon

Host species Rabbit

Tested applications Suitable for: WB, IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide corresponding to Human CD3 epsilon aa 150 to the C-terminus. Synthetic

peptide:

KAKAKPVTRGAGA

, corresponding to amino acids 156-168 of Human CD3 Epsilon chain.

Run BLAST with
Run BLAST with

General notes Abcam is committed to meeting high standards of manufacturing and has decided to discontinue

this product once the stock runs out as we are unable to secure its future high-quality supply. We suggest <u>ab16669</u> as possible replacement. We are sorry for any inconvenience this may case.

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

#### **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

**Storage buffer** pH: 7.40

Preservative: 0.05% Sodium azide

Constituent: PBS

**Purity** Immunogen affinity purified

**Clonality** Polyclonal

**Isotype** IgG

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#### **Applications**

#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab5690 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	* * * * * <u>(5)</u>	Use a concentration of 0.5 - 2 µg/ml. Predicted molecular weight: 23 kDa.
IHC-P	<b>★★★★★</b> (29)	1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. The recommended starting incubation time is 10min.

#### **Target**

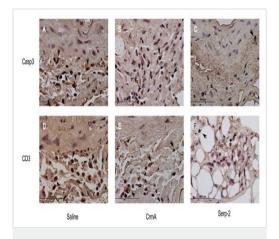
**Function** The CD3 complex mediates signal transduction.

**Sequence similarities** Contains 1 lg-like (immunoglobulin-like) domain.

Contains 1 ITAM domain.

**Cellular localization** Membrane.

#### **Images**

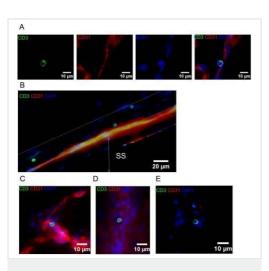


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 epsilon antibody (ab5690)

Viswanathan, K. et al PLoS One. 2012;7(9):e44694. doi: 10.1371/journal.pone.0044694. Epub 2012 Sep 26 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

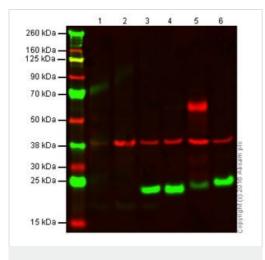
# CD3 and active Caspase 3 populations 72 hrs after mouse aortic allograft

C57Bl/6 donor aortic allografts were transplanted into Balb/C recipient mice (N=3 per treatment) and followed up at 72hrs. Compared to saline, Serp-2 but not CrmA treatment reduced caspase 3 activity (panels A-C; p<0.0224). Neither protein treatment significantly reduced CD3+ T cells (panels D-F).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 epsilon antibody (ab5690)

Divan, A et al LoS One. 2018 May 3;13(5):e0196893. doi: 10.1371/journal.pone.0196893. eCollection 2018 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/



Western blot - Anti-CD3 epsilon antibody (ab5690)

# T cells in central nervous system during late disseminated infection

A-E) Representative epifluorescence images of T cells (CD3), blood vessels (CD31), and nucleated cells (DAPI) in the brain, dura mater, and pia mater. (A) Epifluorescence images described from left to right. CD3 shown in FITC channel; CD31+ blood vessels shown in TRITC channel; nucleated cells shown in DAPI channel; merged image showing CD3+ cell associated with pia mater within the commissure of the isocortex. (B) T cells within the lymphatic-like vascular region of the sagittal sinus in the dura mater. (C) T cell associated with a blood vessel in the vasculature of the brain choroid plexus. (D) T cell associated with blood vessel in the dura mater.

(After Figure 3 of Dirvan et al)

All lanes: Anti-CD3 epsilon antibody (ab5690) at 1 µg/ml

Lane 1: THP1 whole cell lysate (-ve control)

Lane 2: Raji whole cell lysate (-ve control)

Lane 3: Jurkat whole cell lysate

Lane 4: Human Thymus tissue lysate

Lane 5: Mouse Thymus tissue lysate

Lane 6: Rat Thymus tissue lysate

Lysates/proteins at 15 µg per lane.

#### **Secondary**

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

Performed under reducing conditions.

**Predicted band size:** 23 kDa **Observed band size:** 23 kDa

Lanes 1 - 6: Merged signal (red and green). Green - ab5690

observed at 23 kDa. Red - loading control, ab8245, observed at 37

This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using Licor blocking buffer before being incubated with ab5690 and ab8245 (loading control) overnight at 4°C. Antibody binding was detected using Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) at a 1:10000 dilution for 1hr at room temperature and then imaged.

IHC image of CD3 epsilon staining in a formalin fixed, paraffin embedded normal human tonsil tissue section\*, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6) for 20 mins. The section was then incubated with ab5690 at 1/100 dilution for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

supported by the NIHR Cambridge Biomedical Research Centre

\*Tissue obtained from the Human Research Tissue Bank.

Lanes 1-2: Anti-CD3 epsilon antibody (ab5690) at 1 µg/ml

Lanes 3-4: No primary antibody

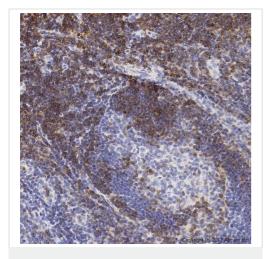
Lanes 1 & 3: Jurkat cell lysate at 30 µg

Lanes 2 & 4: Rat thymus tissue lysate at 20 µg

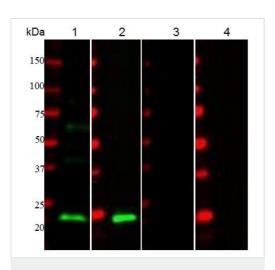
#### Secondary

All lanes: Goat anti-rabbit lgG (H+L), highly cross - adsorbed, HiLyte™ Fluor 750-labeled at 1/12500 dilution

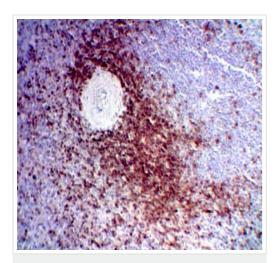
Predicted band size: 23 kDa Observed band size: 23 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 epsilon antibody (ab5690)

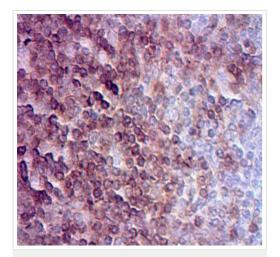


Western blot - Anti-CD3 epsilon antibody (ab5690)



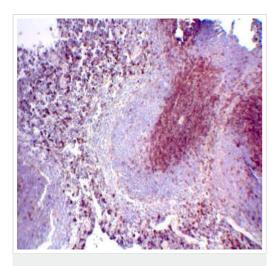
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 epsilon antibody (ab5690)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human spleen tissue labelling CD3 epsilon with ab5690 at 2µl/ml. Slides were steamed in IHC epitope retrieval solution for 35 minutes and then cooled for 20 minutes. Samples were incubated with the primary antibody at room temperature for 1 hour, incubated with a biotinylated secondary antibody for 30 minutes followed by HRP-Streptavidin for 30 minutes. Developed with DAB chromogen substrate for 5-10 minutes. Counter stained with hematoxylin. Magnification: 10X.



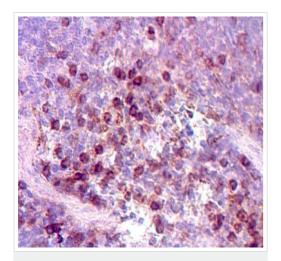
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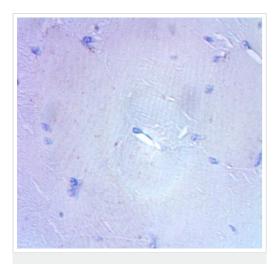
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 epsilon antibody (ab5690)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse spleen tissue labelling CD3 epsilon with ab5690 at 2µl/ml. Slides were steamed in IHC epitope retrieval solution for 35 minutes and then cooled for 20 minutes. Samples were incubated with the primary antibody at room temperature for 1 hour, incubated with a biotinylated secondary antibody for 30 minutes followed by HRP-Streptavidin for 30 minutes. Developed with DAB chromogen substrate for 5-10 minutes. Counter stained with hematoxylin. Magnification: 10X.



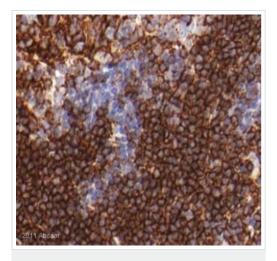
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 epsilon antibody (ab5690)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse spleen tissue labelling CD3 epsilon with ab5690 at 2µl/ml. Slides were steamed in IHC epitope retrieval solution for 35 minutes and then cooled for 20 minutes. Samples were incubated with the primary antibody at room temperature for 1 hour, incubated with a biotinylated secondary antibody for 30 minutes followed by HRP-Streptavidin for 30 minutes. Developed with DAB chromogen substrate for 5-10 minutes. Counter stained with hematoxylin. Magnification: 40X.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 epsilon antibody (ab5690)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human skeletal muscle tissue showing no expression of CD3 epsilon when labelled with ab5690 at 2μl/ml. Slides were steamed in IHC epitope retrieval solution for 35 minutes and then cooled for 20 minutes. Samples were incubated with the primary antibody at room temperature for 1 hour, incubated with a biotinylated secondary antibody for 30 minutes followed by HRP-Streptavidin for 30 minutes. Developed with DAB chromogen substrate for 5-10 minutes. Counter stained with hematoxylin. Magnification: 40X.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 epsilon antibody (ab5690)

This image is courtesy of an Anonymous Abreview.

ab5690 staining CD3 epsilon in human lymph node tissue by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded tissue sections). The sections were fixed in paraformaldehyde and subjected to heat-mediated antigen retrieval in citric buffer pH 6.0, prior to blocking with 10% serum for 1 hour at 20°C. The primary antibody was diluted 1/100 and incubated with the sample for 12 hours at 4°C. An HRP-conjugated goat anti-rabbit polyclonal was used as the secondary antibody, diluted 1/200.

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