## Overview

**Product name**  
Anti-CD3 antibody

**Description**  
Rabbit polyclonal to CD3

**Host species**  
Rabbit

**Tested applications**  
Suitable for: IHC-Fr, WB, IHC-P, IHC-FoFr, ICC/IF

**Species reactivity**  
Reacts with: Mouse, Rat, Human

**Immunogen**  
Synthetic peptide:  
KAKAKPVTRGAGA  
, corresponding to amino acids 156-168 of Human CD3 Epsilon chain.

**Positive control**  
WB: Recombinant Human CD3 epsilon protein (ab114153), Jurkat cell lysate and rat thymus tissue lysate. IHC-P: Human tonsil, lymph node and spleen tissue; mouse spleen tissue rat spleen tissue. IHC-Fr: Mouse lymph node tissue.

**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 ab16669 as possible replacement. We are sorry for any inconvenience this may cause.

## 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

## Applications

Our Abpromise guarantee covers the use of ab5690 in the following tested applications.
The CD3 complex mediates signal transduction.

**Function**

The CD3 complex mediates signal transduction.

**Involvement in disease**

Defects in CD3d are a cause of severe combined immunodeficiency autosomal recessive T-cell-negative/B-cell-positive/NK-cell-positive (T(-)/B(+)/NK(+) SCID) [MIM:608971]. A form of severe combined immunodeficiency (SCID), a genetically and clinically heterogeneous group of rare congenital disorders characterized by impairment of both humoral and cell-mediated immunity, leukopenia, and low or absent antibody levels. Patients present in infancy recurrent, persistent infections by opportunistic organisms. The common characteristic of all types of SCID is absence of T-cell-mediated cellular immunity due to a defect in T-cell development.

**Sequence similarities**

Contains 1 ITAM domain.

**Cellular localization**

Membrane.

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<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/100. PubMed: 18628996</td>
</tr>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 0.5 - 2 µg/ml. Predicted molecular weight: 23 kDa.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. The recommended starting incubation time is 10min.</td>
</tr>
<tr>
<td>IHC-FoFr</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 20126467</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration. PubMed: 23874197</td>
</tr>
</tbody>
</table>

**Target**

**Function**

The CD3 complex mediates signal transduction.

**Involvement in disease**

Defects in CD3D are a cause of severe combined immunodeficiency autosomal recessive T-cell-negative/B-cell-positive/NK-cell-positive (T(-)/B(+)/NK(+) SCID) [MIM:608971]. A form of severe combined immunodeficiency (SCID), a genetically and clinically heterogeneous group of rare congenital disorders characterized by impairment of both humoral and cell-mediated immunity, leukopenia, and low or absent antibody levels. Patients present in infancy recurrent, persistent infections by opportunistic organisms. The common characteristic of all types of SCID is absence of T-cell-mediated cellular immunity due to a defect in T-cell development.

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Contains 1 ITAM domain.

**Cellular localization**

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**Images**

**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 - Anti-CD3 antibody (ab5690)**

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**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. (E) T cell in extravascular region of the dura mater.

(After Figure 3 of Dirvan et al)

**Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody (ab5690)**

IHC image of CD3 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.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre
ab5690 staining CD3 (green) in mouse lymph node tissue sections by Immunohistochemistry (IHC-Fr - frozen sections). Tissue was fixed with acetone/alcohol and blocked with 1% BSA for 1 hour at 23°C. Samples were incubated with primary antibody (1/250 in 1% BSA) for 12 hours at 4°C. An Alexa Fluor® 568-conjugated goat anti-rabbit IgG polyclonal (1/1000) was used as the secondary antibody.

CD8 (red) was labelled with ab22378 followed by an Alexa Fluor®, 647-conjugated goat anti-rabbit IgG.

CD3+ CD8+ are yellow.

**All lanes** : Anti-CD3 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 IgG 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.

Lanes 1-2 : Anti-CD3 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 IgG (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 paraffin-embedded sections) analysis of human spleen tissue labelling CD3 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 paraffin-embedded sections) analysis of human spleen tissue labelling CD3 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.

No positive immunostaining for CD3ε, a pan T cell marker (ab5690), was detected in the corneas of scrambled siRNA-treated mice (A) at 5 days p.i. In contrast, positive immunostaining (red) was observed in the peripheral cornea of HIF-1α silenced animals (B). The control sections shown in (C) and (D) were immunostained with species-specific IgG and were positive for SYTOX Green nuclear stain only. Images shown are representative of three independent experiments each with three mice per group. Magnification=180×; inset=335×.

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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human skeletal muscle tissue showing no expression of CD3 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.

ab5690 staining CD3 in the lymphatic nodule of rat spleen by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Tissue was fixed with formaldehyde and a heat mediated antigen retrieval step was performed using citrate buffer pH 6.0. Samples were then blocked with 1% BSA for 25 minutes at 20°C followed by incubation with the primary antibody at a 1/100 dilution for 1 hour at 20°C. A biotin-conjugated goat anti-rat polyclonal was used undiluted as the secondary antibody. CD3 positive cells are mainly distributed in PALS (T-cell region), while sporadic CD3 positive cells are identified in lymphoid follicle and germinal center (B-cell region).

Image courtesy of an anonymous Abreview.
ab5690 staining CD3 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.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"