# abcam

# Product datasheet

# Anti-CD3 antibody [CD3-12] ab11089

\*\*\* \* 18 Abreviews 85 References 10 Images

Overview

Product name Anti-CD3 antibody [CD3-12]

**Description** Rat monoclonal [CD3-12] to CD3

Host species Rat

**Tested applications** Suitable for: IHC-P, WB, Flow Cyt (Intra)

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Sheep, Rabbit, Horse, Chicken, Cow, Cat, Dog, Pig, Duck,

Cynomolgus monkey, Rhesus monkey, Woodchuck, Raccoon, Alpaca

**Immunogen** Synthetic peptide corresponding to Human CD3. Derived from cytoplasmic epitope.

Database link: P07766

Run BLAST with
Run BLAST with

**Positive control** IHC-P: Human tonsil tissue. Mouse spleen and lymph node tissue; Rat spleen tissue. Flow Cyt

(Intra): Human peripheral blood lymphocytes. WB: Jurkat, MOLT-4 and EL4 cell lysates; Human,

mouse and rat thymus tissue lysates.

**General notes**This product should be stored undiluted. Storage in frost free freezers is not recommended.

Should this product contain a precipitate we recommend microcentrifugation before use.

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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -

80°C. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.09% Sodium azide

Constituent: PBS

**Purity** Protein G purified

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**Purification notes** Purified IgG prepared from tissue culture supernatant.

ClonalityMonoclonalClone numberCD3-12IsotypeIgG1

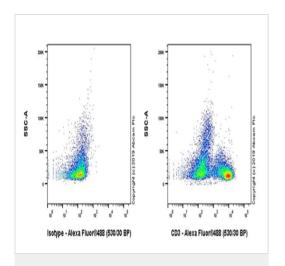
## **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab11089 in the following tested applications.

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

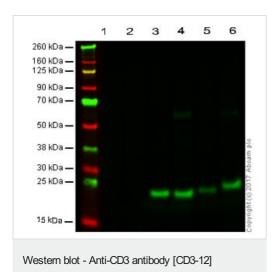
Application	Abreviews	Notes
IHC-P	<b>★★★★★</b> (14)	1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.  1mM EDTA pH8.0 is recommended for this purpose.
WB		Use at an assay dependent concentration. Predicted molecular weight: 23.1 kDa.
Flow Cyt (Intra)		1/50 - 1/100.  Membrane permeabilization is recommended for this application.  ab18407 - Rat monoclonal lgG1, is suitable for use as an isotype control with this antibody.

#### **Images**



Flow Cytometry (Intracellular) - Anti-CD3 antibody [CD3-12] (ab11089)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 0.1% Tween-20 permeabilized human peripheral blood mononuclear cells (PBMC) labeling CD3 with ab11089 at 1/400 dilution (red) compared with a Mouse IgG, monoclonal Isotype Control (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Mouse IgG <u>ab150157</u> (Alexa Fluor<sup>®</sup>488) at 1/2000 dilution was used as the secondary antibody.



(ab11089)

All lanes: Anti-CD3 antibody [CD3-12] (ab11089) at 1 μg/ml

**Lane 1 :** THP-1 (Human monocytic leukemia cell line) whole cell lysate (negative control)

**Lane 2**: Raji (Human Burkitt's lymphoma cell line) whole cell lysate (negative control)

**Lane 3 :** Jurkat (Human T cell leukemia cell line from peripheral blood) 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.

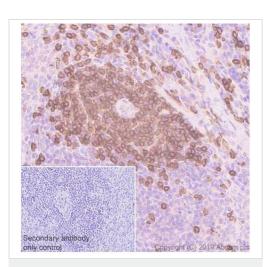
#### Secondary

All lanes: Goat anti-Rat at 1/10000 dilution

Performed under reducing conditions.

**Predicted band size:** 23.1 kDa **Observed band size:** 23 kDa

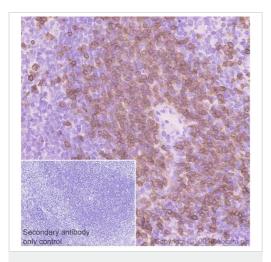
This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using Licor blocking buffer before being incubated with ab11089 overnight at 4°C. Antibody binding was detected using Goat anti-Rat secondary at a 1:10000 dilution for 1hr at room temperature and then imaged.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 antibody [CD3-12] (ab11089)

Immunohistochemical analysis of paraffin-embedded rat spleen tissue labeling CB3 with ab11089 at 1/200 dilution, followed by ready to use Goat Anti-rat IgG H&L (HRP polymer) (ab214882). Positive staining on rat spleen tissue is observed. Counterstained with hematoxylin.

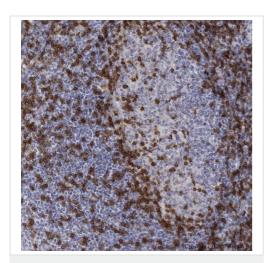
Secondary antibody only control: Used PBS instead of primary antibody, followed by ready to use Goat Anti-Mouse IgG H&L (HRP polymer) (ab214882). Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 antibody [CD3-12] (ab11089)

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling CD3 with ab11089 at 1/200 dilution, followed by ready to use Goat Anti-Rat IgG H&L (HRP polymer) (ab214882). Positive staining on mouse spleen tissue is observed. Counterstained with hematoxylin.

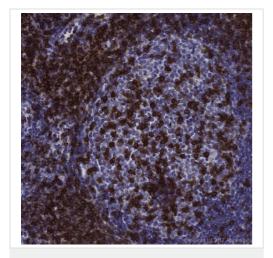
Secondary antibody only control: Used PBS instead of primary antibody, followed by ready to use Goat Anti-Mouse IgG H&L (HRP polymer) (ab214882). Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 antibody [CD3-12] (ab11089)

IHC image of CD3 staining in a formalin fixed, paraffin embedded human tonsil tissue. The section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH 6). The section was incubated with ab11089 at 1/250 dilution for 15 minutes at room temperature. A goat anti-rat biotinylated secondary antibody was used to detect the primary, and visualized using an HRP conjugated ABC system. The section was 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.

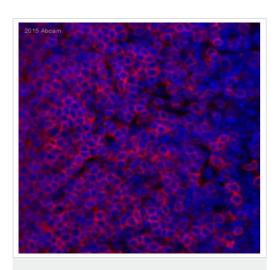


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 antibody [CD3-12] (ab11089)

IHC image of CD3 staining in a formalin fixed, paraffin embedded normal mouse lymph node tissue.

The section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH 6). The section was incubated with ab11089 at 1/250 dilution for 15 minutes at room temperature. A goat anti-rat biotinylated secondary antibody was used to detect the primary, and visualized using an HRP conjugated ABC system. The section was 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.

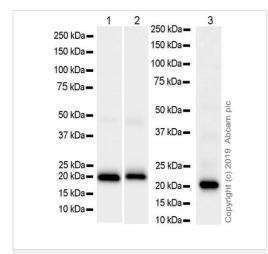


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD3 antibody [CD3-12] (ab11089)

This image is courtesy of an anonymous Abreview.

ab11089 staining CD3 in mouse lymph node tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded sections).

Tissue was fixed with formaldehyde and blocked with 15% serum for 60 minutes at 20°C. Antigen retrieval was by heat mediation in sodium citrate (pH 6). Samples were incubated with primary antibody (1/250 in TBS) for 18 hours at 20°C. An Alexa Fluor<sup>®</sup>conjugated goat anti-rat IgG polyclonal (1/400) was used as the secondary antibody.



Western blot - Anti-CD3 antibody [CD3-12] (ab11089)

**All lanes :** Anti-CD3 antibody [CD3-12] (ab11089) at 1/1000 dilution

Lane 1: Mouse thymus tissue lysate

Lane 2: Rat thymus tissue lysate

Lane 3: Human thymus tissue lysate

Lysates/proteins at 20 µg per lane.

## **Secondary**

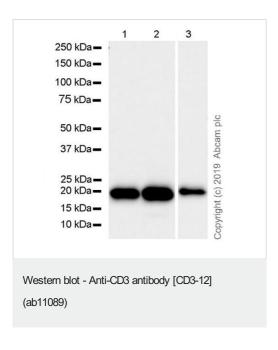
**All lanes :** Goat Anti-Rat lgG H&L (HRP) (<u>ab205720</u>) at 1/5000

dilution

Predicted band size: 23.1 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure: Lanes 1/3: 5 secs; Lanes 2: 3 secs.



**All lanes :** Anti-CD3 antibody [CD3-12] (ab11089) at 1/1000 dilution

Lane 1 : Jurkat (human T cell leukemia T lymphocyte)

Lane 2: MOLT-4 (human lymphoblastic leukemia T lymphoblast)

Lane 3: EL4 (mouse lymphoma T lymphocyte) whole cell lysate

Lysates/proteins at 20 µg per lane.

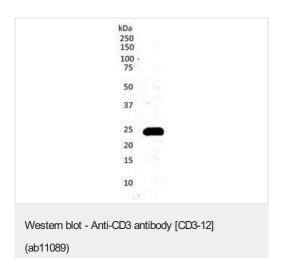
#### Secondary

**All lanes :** Goat Anti-Rat lgG H&L (HRP) (<u>ab205720</u>) at 1/5000 dilution

Predicted band size: 23.1 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 5 secs.



Anti-CD3 antibody [CD3-12] (ab11089) + Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

#### Secondary

HRP-conjugated goat anti-rat lgG

Predicted band size: 23.1 kDa

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

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