abcam

Product datasheet

Anti-CD3 antibody [CD3-12] - BSA and Azide free ab255972

Recombinant

1 Abreviews 9 Images

Overview

Product name	Anti-CD3 antibody [CD3-12] - BSA and Azide free
Description	Rat monoclonal [CD3-12] to CD3 - BSA and Azide free
Host species	Rat
Tested applications	**Suitable for:** IHC-P, WB, Flow Cyt
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human tonsil tissue. Mouse spleen and lymph node tissue; Rat spleen tissue. Flow Cyt: Human peripheral blood lymphocytes. WB: Jurkat, MOLT-4 and EL4 cell lysates; Human, mouse and rat thymus tissue lysates.
General notes	ab255972 is the carrier-free version of **ab11089**. This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	CD3-12
Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab255972 in the following tested applications.

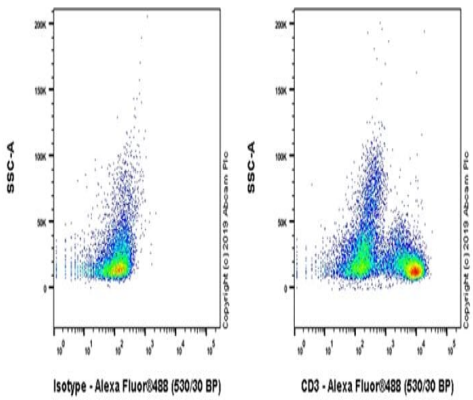
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P	★★★★☆ **(1)**	1/200. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Predicted molecular weight: 19 kDa.
Flow Cyt		1/400.

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.
Sequence similarities	Contains 1 ITAM domain.
Cellular localization	Membrane.

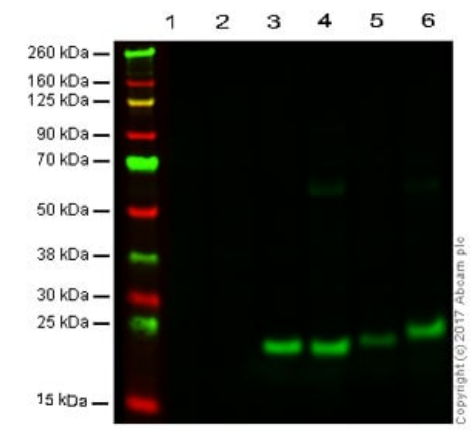
Images



Flow Cytometry - Anti-CD3 antibody [CD3-12] - BSA and Azide free (ab255972)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab11089**).

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 **ab150157** (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-CD3 antibody [CD3-12] - BSA and Azide free (ab255972)

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

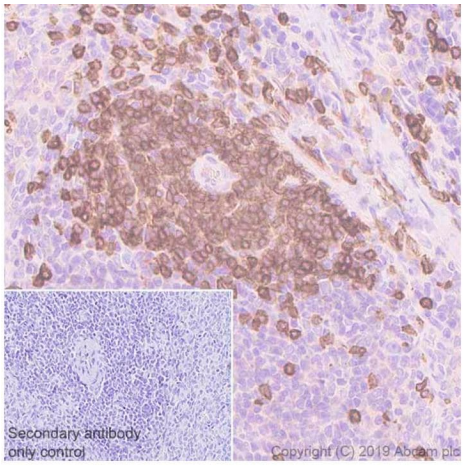
Performed under reducing conditions.

Predicted band size: 19 kDa
Observed band size: 23 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab11089**).

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.

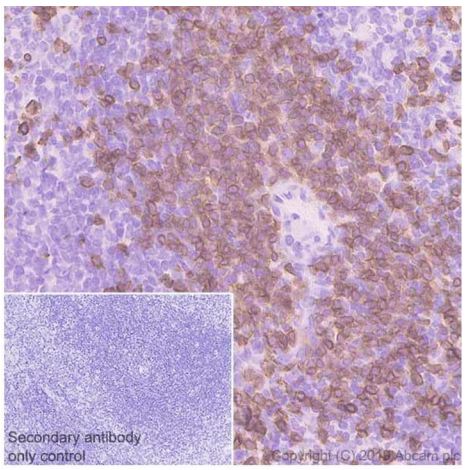


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [CD3-12] - BSA and Azide free (ab255972)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**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).

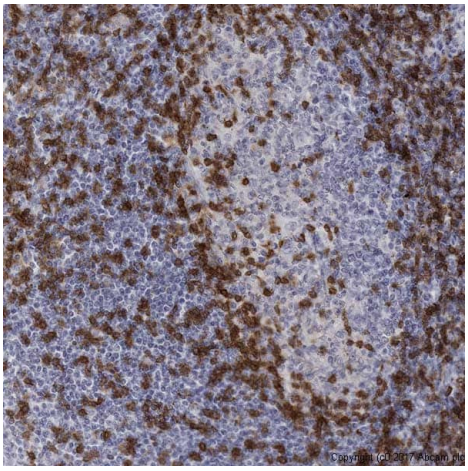


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [CD3-12] - BSA and Azide free (ab255972)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**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).

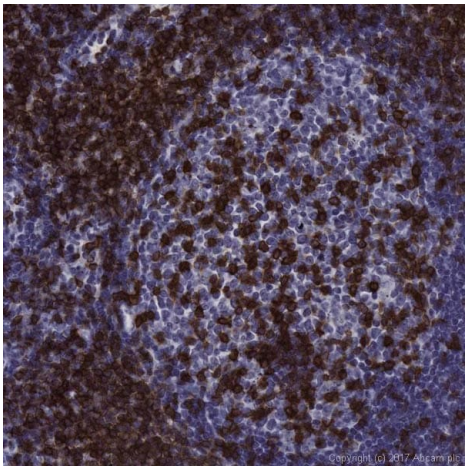


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [CD3-12] - BSA and Azide free (ab255972)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**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.

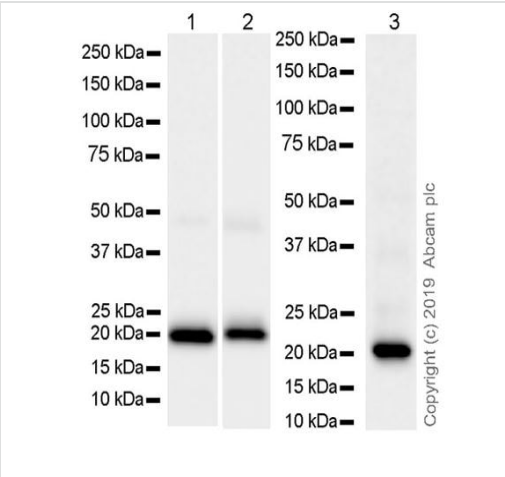


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [CD3-12] - BSA and Azide free (ab255972)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**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.



Western blot - Anti-CD3 antibody [CD3-12] - BSA and Azide free (ab255972)

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.

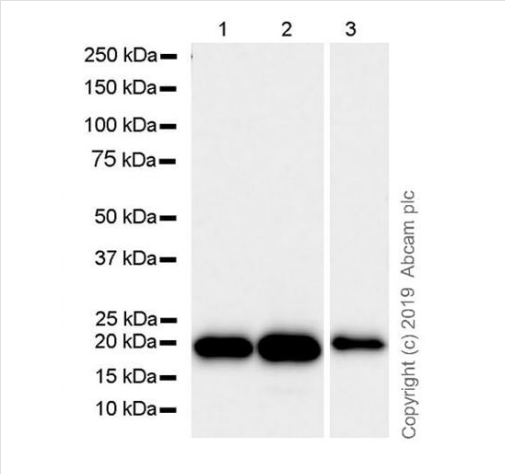
Secondary
All lanes : Goat Anti-Rat IgG H&L (HRP) (**ab205720**) at 1/5000 dilution

Predicted band size: 19 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab11089**).

Blocking and dilution buffer: 5% NFDM/TBST.

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



Western blot - Anti-CD3 antibody [CD3-12] - BSA and Azide free (ab255972)

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 10 µg per lane.

Secondary
All lanes : Goat Anti-Rat IgG H&L (HRP) (**ab205720**) at 1/5000 dilution

Predicted band size: 19 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab11089**).

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 5 secs.

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