abcam

Product datasheet

Anti-CD3 antibody [SP7], prediluted ab21703

Recombinant RabMAb

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Overview

Product name	Anti-CD3 antibody [SP7], prediluted
Description	Rabbit monoclonal [SP7] to CD3, prediluted
Host species	Rabbit
Specificity	ab21703 recognises CD3 epsilon chain. This antibody reacts with the intracytoplasmic portion of the CD3 antigen expressed by T cells. It stains human T cells in both the cortex and medulla of the thymus and in peripheral lymphoid tissues.
Tested applications	**Suitable for:** WB, mIHC, Flow Cyt (Intra), IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Sheep, Rabbit, Cow, Dog, Pig, Cynomolgus monkey, Macaque monkey, Woodchuck
Immunogen	Synthetic peptide within Human CD3 aa 150 to the C-terminus. The exact sequence is proprietary. Database link: **P07766**
Positive control	Tonsil tissue. Recombinant Human CD3 epsilon protein (**ab114153**) can be used as a positive control in WB.
General notes	This antibody is suitable for staining normal and neoplastic T cells. 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**. **This product is FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.**

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C.

Storage buffer	pH: 7.2 Preservative: 0.1% Sodium azide Constituents: 1% BSA, PBS Inert stabilizer
Purity	Protein A purified
Primary antibody notes	This antibody is suitable for staining normal and neoplastic T cells.
Clonality	Monoclonal
Clone number	SP7
Isotype	IgG

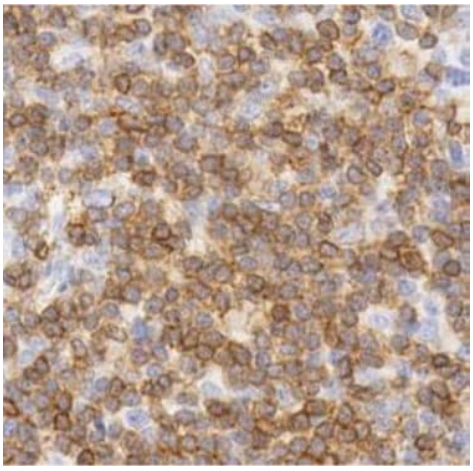
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab21703 in the following tested applications.

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

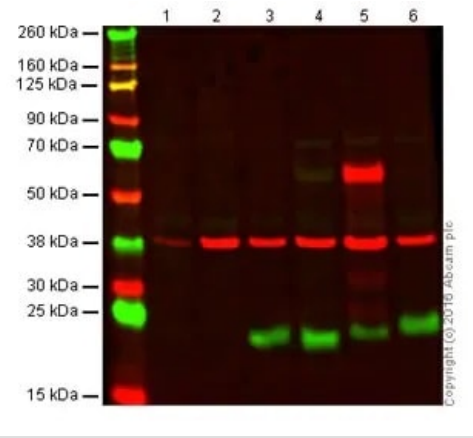
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 19 kDa.
mIHC		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
IHC-P	★★★★★ (1)	1/150. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. Use neat. Perform high temperature antigen unmasking with 10 mM citrate buffer, pH 6.0.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD3 antibody [SP7], prediluted (ab21703)

Immunohistochemical analysis of paraffin-embedded Human tonsil labeling CD3 with ab21703 at 1/150 (7 µg/ml). The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab21703 for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. DAB was used as the chromogen. Counterstained with Hematoxylin and mounted with DPX.



Western blot - Anti-CD3 antibody [SP7], prediluted (ab21703)

All lanes : Anti-CD3 epsilon antibody [SP7] (**ab16669**) at 1/25 dilution

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

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

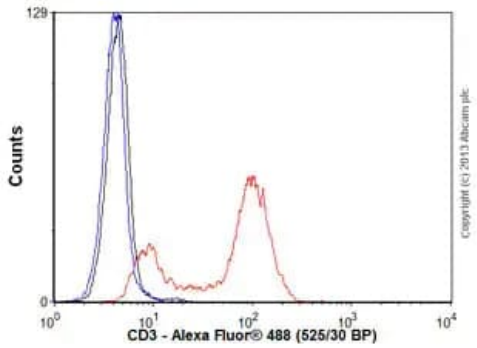
This image was generated using a previous batch manufactured using hybridoma production method.

Lanes 1 - 6: Merged signal (red and green). Green – **ab16669** observed at 23 kDa. Red - loading control, **ab8245**, observed at 37 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 **ab16669** and **ab8245** (loading control) overnight at 4°C. Antibody binding was detected using Goat **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.

This data was developed using the undiluted version of this antibody (**ab16669**).

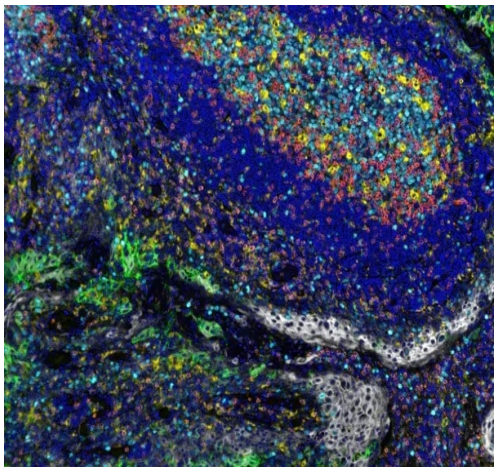


Flow Cytometry (Intracellular) - Anti-CD3 antibody [SP7], prediluted (ab21703)

This image was generated using a previous batch manufactured using hybridoma production method.

Human peripheral blood lymphocytes stained with **ab16669** (red line). Human whole blood was processed using a modified protocol based on Chow *et al*, 2005 (PMID: 16080188). In brief, human whole blood was fixed in 4% formaldehyde (methanol-free) for 10 min at 22°C. Red blood cells were then lyzed by the addition of Triton X-100 (final concentration - 0.1%) for 15 min at 37°C. For experimentation, cells were treated with 50% methanol (-20°C) for 15 min at 4°C. Cells were then incubated with the antibody (**ab16669**, 1/1000 dilution) for 30 min at 4°C. The secondary antibody used was **Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody** at 1/2000 dilution for 30 min at 4°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >30,000 total events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. Gating strategy - peripheral blood lymphocytes.

This data was developed using the undiluted version of this antibody (**ab16669**).



Multiplex immunohistochemistry - Anti-CD3 antibody [SP7], prediluted (ab21703)

This image was generated using a previous batch manufactured using hybridoma production method.

This data was developed using the undiluted version of this antibody (**ab16669**).

Fluorescence multiplex immunohistochemical analysis of normal human tonsil tissue (formalin-fixed paraffin-embedded section).

Merged staining of anti-PD1 (**ab237728**; orange; Opal™520), anti-PDL1 (**ab237726**; green; Opal™540), anti-CD68 (**ab192847**; yellow; Opal™570), anti-CD3 (**ab16669**; red; Opal™620), anti-Ki67 (**ab16667**; light blue; Opal™650) and anti-PanCK (**ab7753**; grey; Opal™690).

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 7-color automation IHC kit (NEL821001KT, Akoya Biosciences®).

The section was incubated in six rounds of staining; in the order of **ab237728** (1/500 dilution), **ab237726** (1/500 dilution), **ab192847** (1/300 dilution), **ab16669** (1/300 dilution), **ab16667** (1/200 dilution) and **ab7753** (1/200 dilution); each using a separate fluorescent tyramide signal amplification system.

Sodium citrate antigen retrieval (Leica ER1, pH6.0, 30 minutes) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity.

DAPI (dark blue) was used as a nuclear counter stain.

Microscopy and pseudocoloring of individual Opal™ dyes was performed using a Vectra Polaris.

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