abcam

Product datasheet

Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y] ab68236

Recombinant RabMAb

11 References 5 Images

Overview

Product name	Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y]
Description	Rabbit monoclonal [EP776(2)Y] to CD3 zeta (phospho Y83)
Host species	Rabbit
Specificity	This antibody detects CD3 zeta phosphorylated on tyrosine 83.
Tested applications	**Suitable for:** Flow Cyt, ICC/IF, Dot blot, WB, IP **Unsuitable for:** IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human CD3 zeta aa 50-150 (phospho Y83). The exact sequence is proprietary. Database link: P20963
Positive control	WB: Jurkat whole cell lysate (ab7899).
General notes	Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information. 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. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents. **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.**

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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.05% Sodium azide Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP776(2)Y
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab68236** in the following tested applications.

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

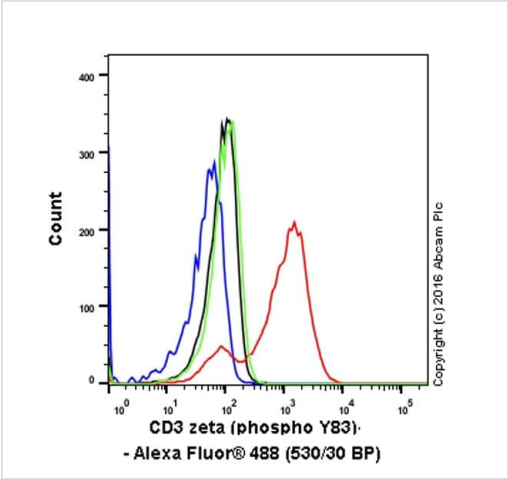
Application	Abreviews	Notes
Flow Cyt		Use at an assay dependent concentration.
ICC/IF		1/100 - 1/250.
Dot blot		Use at an assay dependent concentration.
WB		1/5000 - 1/10000. Detects a band of approximately 18-22 kDa (predicted molecular weight: 18 kDa).
IP		1/50.

Application notes	Is unsuitable for IHC-P.

Target

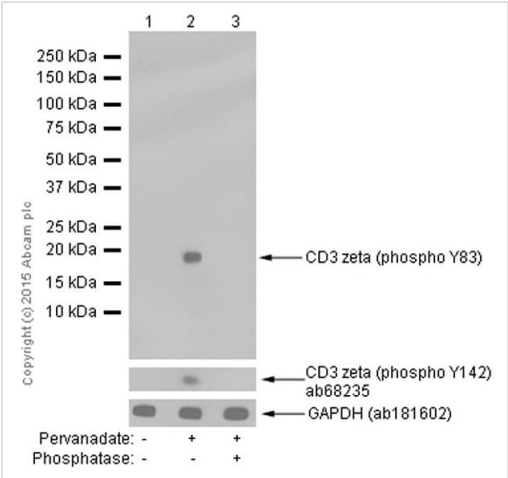
Function	Probable role in assembly and expression of the TCR complex as well as signal transduction upon antigen triggering.
Involvement in disease	Defects in CD247 are the cause of immunodeficiency due to defect in CD3-zeta (CD3ZID) [MIM:610163]. An immunological deficiency characterized by T-cells impaired immune response to alloantigens, tetanus toxoid and mitogens.
Sequence similarities	Belongs to the CD3Z/FCER1G family. Contains 3 ITAM domains.
Domain	The ITAM domains mediate interaction with SHB.
Post-translational modifications	Phosphorylated on Tyr residues after T-cell receptor triggering.
Cellular localization	Membrane.

Images



Flow Cytometry - Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y] (ab68236)

Flow Cytometry analysis of Jurkat (human acute T cell leukemia) treated (Red)/untreated (Green) with 1mM pervanadate for 4 hours with purified ab68236 at 1/250 dilution. The secondary antibody was Goat anti rabbit IgG (Alexa Fluor® 488) at 1/2000 dilution. A Rabbit monoclonal IgG (Black) was used as the isotype control and cells without incubation with primary antibody and secondary antibody (Blue) were used as unlabeled control.



Western blot - Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y] (ab68236)

All lanes : Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y] (ab68236) at 1/2000 dilution

Lane 1 : Untreated Jurkat cells whole cell lysates
Lane 2 : Jurkat cells were treated with 50mM Pervanadate for 5 minutes whole cell lysates
Lane 3 : Jurkat cells were treated with 50mM Pervanadate for 5 minutes whole cell lysates. Then the membrane was incubated with Alkaline phosphatase.

Lysates/proteins at 10 µg per lane.

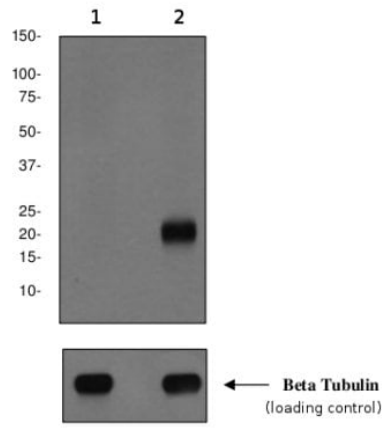
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 18 kDa
Observed band size: 18 kDa

Exposure time: 3 minutes

Blocking buffer 5% NFDM/TBST

Diluting buffer 5% NFDM/TBST



Western blot - Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y] (ab68236)

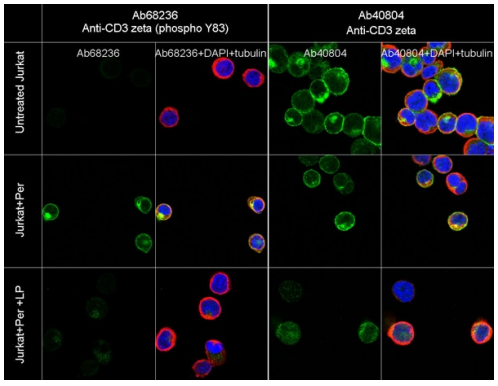
All lanes : Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y] (ab68236) at 1/10000 dilution

Lane 1 : Jurkat cell lysate, untreated.
Lane 2 : Jurkat cell lysate, treated with pervanadate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution

Predicted band size: 18 kDa
Observed band size: 18-22 kDa
why is the actual band size different from the predicted?

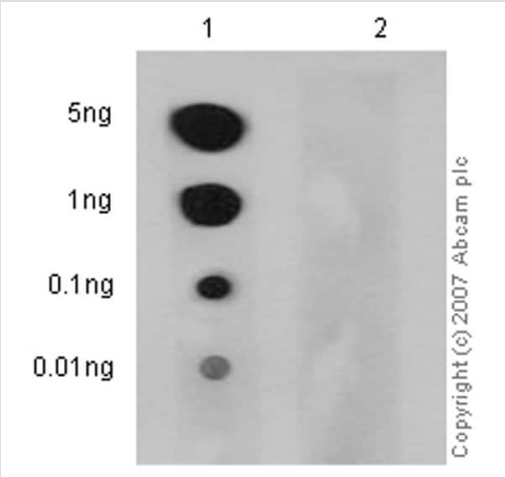


Immunocytochemistry/ Immunofluorescence - Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y] (ab68236)

Immunocytochemistry/Immunofluorescence analysis of Jurkat cells (untreated, Per treated and Per+LP treated) labelling CD3 zeta (phospho Y83) with ab68236 (left) and CD3 zeta with ab40804 (right) both at a dilution of 1/200. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse anti-tubulin (1/1000) and ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used.

The image shows increased cytoplamic staining after Pervanadate (1 mM, 30 min) treatment on Jurkat cells. The LP treatment decreased the cytoplasmic staining caused by Pervanadate.

ab40804 was used as a Pan control for ab68236. The results showed cytoplamic staining on untreated, pervanadate (1 mM, 30 min) treated and Per+LP treated Jurkat cells.



Dot Blot - Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y] (ab68236)

Dot blot analysis of CD3 zeta (pY83) phospho peptide (lane 1) and CD3 zeta non-phospho peptide (lane 2) labelling CD3 zeta (phospho Y83) with ab68236 at a dilution of 1/1000. A peroxidase-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/2500).

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

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