abcam

Product datasheet

Anti-CD3 zeta (phospho Y142) antibody [EP265(2)Y] ab68235

Recombinant RabMAb

* ★ ★ ★ ★ 1 Abreviews 9 References 5 Images

Overview

Product name Anti-CD3 zeta (phospho Y142) antibody [EP265(2)Y]

Description Rabbit monoclonal [EP265(2)Y] to CD3 zeta (phospho Y142)

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), ICC/IF, WB, IP

Species reactivity Reacts with: Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control Jurkat whole cell lysate (ab7899)

General notesThis 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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

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 long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

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Clone number EP265(2)Y

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab68235 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 (Intra)		1/120.
ICC/IF	*****(1)	1/250 - 1/500.
WB		1/500 - 1/2000. Detects a band of approximately 21 kDa (predicted molecular weight: 19 kDa).
IP		1/20 - 1/50.

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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 similaritiesBelongs to the CD3Z/FCER1G family.

Contains 3 ITAM domains.

Domain The ITAM domains mediate interaction with SHB.

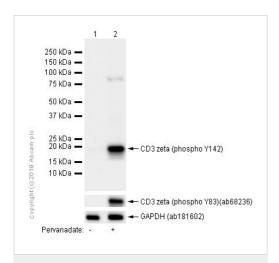
Post-translational

modifications

Phosphorylated on Tyr residues after T-cell receptor triggering.

Cellular localization Membrane.

Images



Western blot - Anti-CD3 zeta (phospho Y142) antibody [EP265(2)Y] (ab68235)

All lanes : Anti-CD3 zeta (phospho Y142) antibody [EP265(2)Y] (ab68235) at 1/1000 dilution (Purified)

Lane 1: Jurkat (Human T cell leukemia T lymphocyte) whole cell lysates

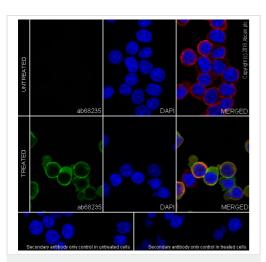
Lane 2 : Jurkat (Human T cell leukemia T lymphocyte) treated with 50mM pervanadate for 5 minutes whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

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

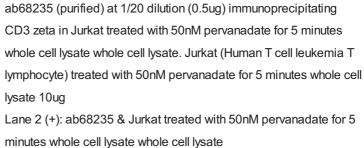


Immunocytochemistry/ Immunofluorescence - Anti-CD3 zeta (phospho Y142) antibody [EP265(2)Y] (ab68235)

Immunocytochemistry/ Immunofluorescence analysis of Jurkat (Human T cell leukemia T lymphocyte) treated with 1mM pervanadate for 30 minutes cells labeling CD3 zeta with purified ab68235 at 1/500 dilution (0.2 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1:200 (2.5 µg/ml). Goat anti rabbit lgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunoprecipitation - Anti-CD3 zeta (phospho Y142) antibody [EP265(2)Y] (ab68235)

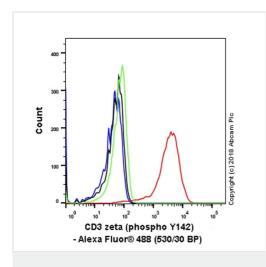


Lane 2 (+): ab68235 & Jurkat treated with 50nM pervanadate for 5

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab68235 in Jurkat treated with 50nM pervanadate for 5 minutes whole cell

For western blotting, VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/2000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-CD3 zeta (phospho Y142) antibody [EP265(2)Y] (ab68235)

Intracellular Flow Cytometry analysis of Jurkat (Human T cell leukemia T lymphocyte) treated with 50nM pervanadate for 5 minutes cells labeling CD3 zeta with purified ab68235 at 1/120 dilution (1µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluorr® 488, ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue). Untreated Jurkat cells (Green).



(ab68235)

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