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

Specificity: This antibody detects CD3 zeta phosphorylated on tyrosine 142.

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

Species reactivity: Reacts with: Human

Immunogen: corresponding to Human CD3 zeta aa 100-200.

Positive control: Jurkat whole cell lysate (ab7899)

General notes:
- 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.

Form: Liquid

Avoid freeze / thaw cycle.

Storage buffer:
- pH: 7.40
- Preservative: 0.01% Sodium azide
- Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity: Protein A purified

Clonality: Monoclonal

Clone number: EP265(2)Y

Isotype: IgG

Applications

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.

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<td>Flow Cyt</td>
<td>1/120.</td>
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<td>WB</td>
<td>1/500 - 1/2000. Detects a band of approximately 21 kDa (predicted molecular weight: 19 kDa).</td>
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<td>IP</td>
<td>1/20 - 1/50.</td>
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<tr>
<td>ICC/IF</td>
<td>1/250 - 1/500.</td>
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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
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 IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 19 kDa

Observed band size: 19 kDa

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 IgG (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.
**Western blot - Anti-CD3 zeta (phospho Y142) antibody [EP265(2)Y]** (ab68235) at 1/1000 dilution (unpurified)

**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:** 19 kDa

**Observed band size:** 18 kDa

**why is the actual band size different from the predicted?**

**Exposure time:** 3 minutes

Blocking buffer 5% NFDM/TBST

Diluting buffer 5% NFDM/TBST
**Immunoprecipitation - Anti-CD3 zeta (phospho Y142) antibody [EP265(2)Y] (ab68235)**

ab68235 (purified) at 1/20 dilution (0.5ug) immunoprecipitating CD3 zeta in Jurkat treated with 50nM pervanadate for 5 minutes whole cell lysate whole cell lysate. Jurkat (Human T cell leukemia T lymphocyte) treated with 50nM pervanadate for 5 minutes whole cell lysate 10ug

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

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

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

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 (1 µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 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).

**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 1/2000 dilution (unpurified)

Lane 1: Jurkat cell lysates, membrane un-treated

Lane 2: Jurkat cell lysates, membrane treated with pervanadate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 19 kDa

Observed band size: 19 kDa
Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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