abcam

Product datasheet

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

ועלשעבע RabMAb

14 References 画像数7

製品の概要

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

製品の詳細 Rabbit monoclonal [EP776(2)Y] to CD3 zeta (phospho Y83)

由来種 Rabbit

アプリケーション 適用あり: Flow Cyt (Intra), ICC/IF, Dot blot, WB, IP

適用なし: IHC-P

種交差性 交差種: Human

免疫原 Synthetic peptide within Human CD3 zeta aa 50-150 (phospho Y83). The exact sequence is

proprietary.

Database link: P20963

ポジティブ・コントロール

WB: Jurkat whole cell lysate (ab7899). IP: Jurkat. ICC: Jurkat cells. Flow Cyt (intra): Jurkat cells. 特記事項

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 $\mathsf{RabMAb}^{\texttt{®}}$ 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.

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

バッファー pH: 7.2

Preservative: 0.05% Sodium azide

Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% PBS

精製度 Protein A purified

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ポリÆノ モノクローナル **ウローン名** EP776(2)Y **アイソタイプ** lgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab68236の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

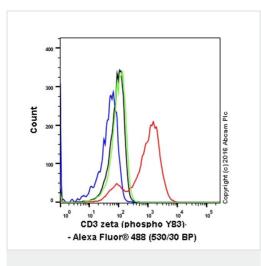
アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF		1/100 - 1/250.
Dot blot		Use at an assay dependent concentration.
WB		1/5000 - 1/10000. Detects a band of approximately 18-22 kDa (predicted molecular weight: 18 kDa).
IP		1/50.

追加情報 Is unsuitable for IHC-P.

ターゲット情報

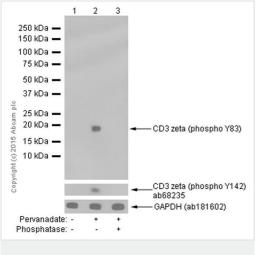
機能 Probable role in assembly and expression of the TCR complex as well as signal transduction upon antigen triggering. 関連疾患 Defects in CD247 are the cause of immunodeficiency due to defect in CD3-zeta (CD3ZID) [MIM:610163]. An immunological deficiency characterized by T-cells impaired immune response to alloantigens, tetanus toxoid and mitogens. 配列類似性 Belongs to the CD3Z/FCER1G family. Contains 3 ITAM domains. ドメイン The ITAM domains mediate interaction with SHB. 翻訳後修飾 Phosphorylated on Tyr residues after T-cell receptor triggering. 細胞内局在 Membrane.

画像



Flow Cytometry (Intracellular) - 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 lgG (Alexa Fluor[®] 488) at 1/2000 dilution. A Rabbit monoclonal lgG (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

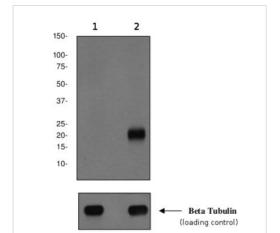
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

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.

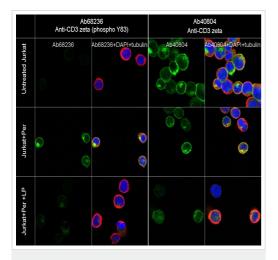
Lane 2: Jurkat cell lysate, treated with pervanadate

Lysates/proteins at 10 µg per lane.

Secondary

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

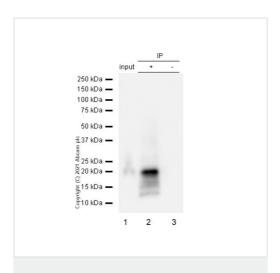
Predicted band size: 18 kDa **Observed band size:** 18-22 kDa



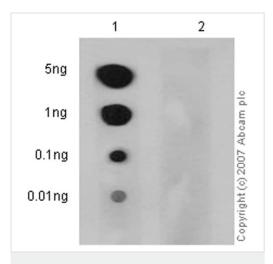
Immunocytochemistry/ Immunofluorescence - Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y] (ab68236) Immunocytochemistry/Immunofluorescence analysis of Jurkat cells (untreated, Per treated and Per+LP treated) labelling CD3 zeta (phospho Y83) with ab68236 (left) and CD3 zeta with ab40804 (right) both 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 lgG (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 lgG (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.

<u>ab40804</u> 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.



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



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

CD3 zeta was immunoprecipitated from 0.35 mg Jurkat (Human T cell leukemia T lymphocyte) treated with pervandate (50mM 5min) whole cell lysate 10 μ g with ab68236 at 1/30 dilution (2 μ g) . VeriBlot for IP Detection Reagent (HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: Jurkat (Human T cell leukemia T lymphocyte) treated with pervandate (50mM 5min) whole cell lysate 10 µg

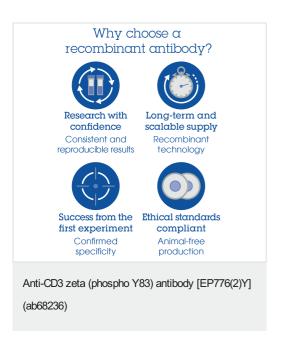
Lane 2: ab68236 IP in Jurkat treated with pervandate (50mM 5min) whole cell lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab68236 in Jurkat treated with pervandate (50mM 5min) whole cell lysate Blocking and dilution buffer and concentration: 5% NFDM/TBST.

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 lgG (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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