abcam

ab68236

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

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.05% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 0.1% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EP776(2)Y
Purification technique	Affinity purification Protein A
Concentration	0.12 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/50
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Dot

Tested

Species	Synthetic peptide
Dilution info	-
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human
Dilution info	1/5000 - 1/10000
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Human, Synthetic peptide
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100 - 1/250
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Target data

See full target information CD247 pY83

Function	Part of the TCR-CD3 complex present on T-lymphocyte cell surface that plays an essential role in adaptive immune response. When antigen presenting cells (APCs) activate T-cell receptor (TCR), TCR-mediated signals are transmitted across the cell membrane by the CD3 chains CD3D, CD3E, CD3G and CD247/CD3Z. All CD3 chains contain immunoreceptor tyrosine-based activation motifs (ITAMs) in their cytoplasmic domain. Upon TCR engagement, these motifs become phosphorylated by Src family protein tyrosine kinases LCK and FYN, resulting in the activation of downstream signaling pathways (PubMed:1384049, PubMed:1385158, PubMed:2470098, PubMed:7509083). CD247/CD3Z ITAMs phosphorylation creates multiple docking sites for the protein kinase ZAP70 leading to ZAP70 phosphorylation and its conversion into a catalytically active enzyme (PubMed:7509083). Plays an important role in intrathymic T-cell differentiation. Additionally, participates in the activity-dependent synapse formation of retinal ganglion cells (RGCs) in both the retina and dorsal lateral geniculate nucleus (dLGN) (By similarity).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Species reactivity
Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

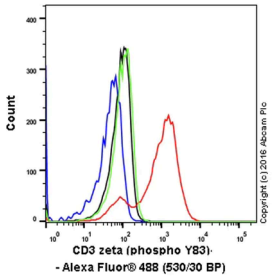
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

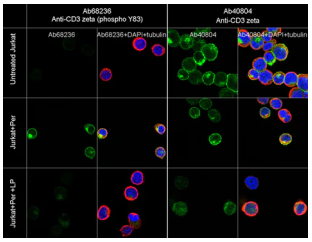
10 product images



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

CD3 zeta (phospho Y83) Flow Cytometry (Intracellular) staining using rabbit Anti-CD3 zeta (phospho Y83) antibody

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.

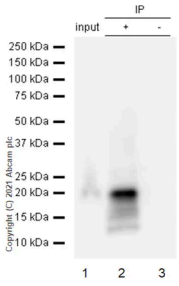


Immunocytochemistry/ Immunofluorescence - Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y] (ab68236)

CD3 zeta (phospho Y83) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-CD3 zeta (phospho Y83) antibody

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.



Immunoprecipitation - 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 µg with ab68236 at 1/30 dilution (2µg) . 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

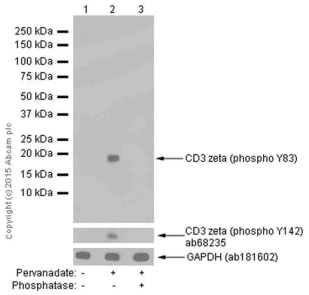
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab68236 in Jurkat treated with pervandate (50mM 5min) whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

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

Predicted band size: 19 kDa

Observed band size: 18-22 kDa



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

CD3 zeta (phospho Y83) Western blot staining using rabbit Anti-CD3 zeta (phospho Y83) antibody

Blocking buffer 5% NFDM/TBST
Diluting buffer 5% NFDM/TBST

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

Lane 1:
Untreated Jurkat cells whole cell lysates at 10 µg

Lane 2:
Jurkat cells were treated with 50mM Pervanadate for 5 minutes whole cell lysates at 10 µg

Lane 3:
Jurkat cells were treated with 50mM Pervanadate for 5 minutes whole cell lysates, then the membrane was incubated with Alkaline phosphatase at 10 µg

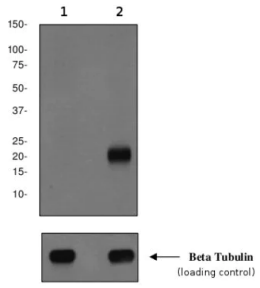
Secondary

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

Predicted band size: 19 kDa

Observed band size: 18 kDa

Exposure time: 3min



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

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

Lane 1:
Jurkat cell lysate, untreated. at 10 µg

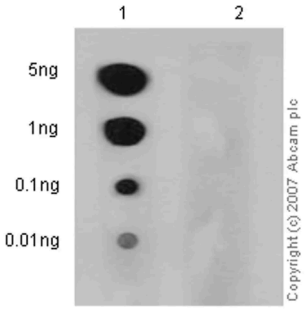
Lane 2:
Jurkat cell lysate, treated with pervanadate at 10 µg

Secondary

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

Predicted band size: 19 kDa

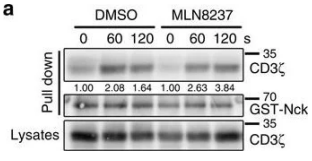
Observed band size: 18-22 kDa



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.



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

CD3 zeta (phospho Y83) Western blot staining using rabbit Anti-CD3 zeta (phospho Y83) antibody

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Western Blotting using Recombinant Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y], ab68236. Publication image from Jorge, I. et al., 2016, Nat Commun, 27091106. Legend direct from paper.

Aurora A inhibition does not affect actin cytoskeleton dynamics.(a) Immunoblot of a pull-down assay of GST-Nck fusion protein from cell lysates of control (DMSO; vehicle) or Aurora A inhibitor (MLN8237)-pretreated human T lymphoblasts. Activation was performed with soluble anti-CD3☒ antibodies for indicated times. CD3ζ and GST are shown. CD3ζ content in whole-cell lysates is indicated in the bottom row. (b) Immunoblotting of Rac1 pull-down assay of GST and GST-PAK-CD from cell lysates of DMSO- or MLN8237-pretreated Jurkat T cells activated with SEE-pulsed Raji B cells (APCs) for the indicated times. Loading control for Rac1 in whole-cell lysates is shown. (c) Images from TIRFm time-lapse analysis of mCherry–β-actin-expressing Jurkat T cells spreading over anti-CD3/CD28-coated glass-bottom chambers. Cells were pretreated with DMSO or MLN8237. Images were taken every 100 ms for 5 min at 90 nm penetrance. A corresponding bright-field image is shown. Scale bar, 10 µm. (d) Quantification of the area occupied by the whole cell (lamella), the actin-rich area (peripheral SMAC (pSMAC)), the central area (cSMAC) and the distribution of mean fluorescence intensity per area (ratios cSMAC:cell; pSMAC:cell and cSMAC/pSMAC) from cells in c (n=48 and n=36, three independent experiments). Cells were fixed after spreading (4 min) and fluorescence images were taken. Data represent means±s.d.; t-test. n.s., nonsignificant. (e) Maximum Z projections of confocal stacks from DMSO- or MLN8237-pretreated Jurkat T cells conjugated with SEE-APCs. Cells were incubated for 30 min, fixed and stained forα-tubulin (green) or actin (magenta). The right-hand image shows CMAC cell tracker labelling of APCs (cyan) and bright field. Scale bar, 10 µm. (f) Quantification of actin accumulation at the IS contact area in conjugates as in e from three independent experiments (n=100). Data represent means±s.d.; t-test. (g) Image sequence for IS formation between mCherry–β-actin-expressing T cells and SEB-APCs (DMSO- or MLN8237-treated). XYZ stacks were acquired every 25 s (maximal projections of XYZ stacks and 3D reconstructions with Imaris Software are shown from representative conjugates). (h) Ratio of T cells forming lamella on contact with an APC from g. Data represent median±interquartile range. Mann–Whitney test (DMSO: 28 cells (n=4); MLN8237: 25 cells (n=3)).

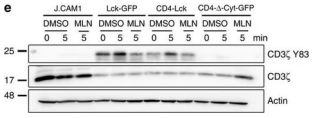


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Western blot - Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y] (ab68236)

CD3 zeta (phospho Y83) Western blot staining using rabbit Anti-CD3 zeta (phospho Y83) antibody

Western Blotting using Recombinant Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y], ab68236. Publication image from Jorge, I. et al., 2016, Nat Commun, 27091106. Legend direct from paper.

Aurora controls localization and phosphorylation of the tyrosine kinase Lck.(a) Maximum Z projection of XYZ stack of human Jurkat T cells pretreated with vehicle (DMSO) or Aurora A inhibitor (MLN8237) and conjugated with SEE-preloaded Raji B cells (APCs; 30 min). Cells were fixed and stained forα-tubulin–fluorescein isothiocyanate (FITC) (green), PKCθ (magenta) and Lck (red). Bright-field images are included. Scale bar, 10 µm. (b) Quantification of Lck accumulation at the IS in conjugates as in a from three independent experiments (DMSO, n=96. MLN8237, n=94). Data represent means±s.d. Means were compared with a t-test; ****P<0.0001. (c) Immunoblotting of Lck phosphorylation at Y394 in primary human CD4+ T cells. Cells were pretreated with DMSO or MLN8237 and conjugated for the indicated times with anti-CD3/CD28-coated beads. Total Lck and actin are included as loading controls. Arrows point Lck band. (d) Quantification of data from four independent experiments as in c. Error bars represent s.d. Medians were compared with a Friedman test (*P<0.05). (e) Immunoblots of CD3ζ phosphorylation in lysates of J.CAM1 T cells transfected with Lck-GFP, CD4-Lck or CD4-δCyt-GFP, pretreated with DMSO or MLN8237 and conjugated for 5 min with SEE-pulsed APCs. (f) T-cell lymphoblasts pretreated with DMSO or MLN8237 were activated or not with SEE-pulsed APCs (2 min) and subjected to IP using an anti-Lck antibody. The immunoprecipitates were subjected to MS analysis. Upper panel, MS/MS extracted ion chromatograms of the Y394-phosphorylated and non-modified forms of Lck peptide LIEDNEYTAR. Lower panel, phosphorylated:non-modified peak ratios. (g) Recombinant Lck was incubated with Aurora A WT (in the absence or presence of MLN8237) or Aurora A KD

immunoprecipitated from nocodazole-treated (16 h), transfected HEK293 cells. Lck and Aurora were incubated for 30 min in the presence of ATP and the mixture analysed by MS. Upper panel, MS/MS extracted ion chromatograms of the Y394-phosphorylated and non-modified forms of Lck peptide LIEDNEYTAR. Lower panel, phosphorylated:non-modified peak ratios. See Supplementary Table 1 for representative MS/MS spectra of the phosphorylated and non-phosphoryated forms of the peptide at the peaks.

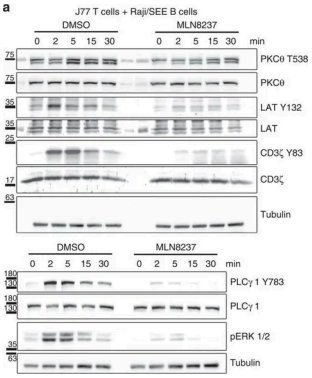


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Western blot - Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y] (ab68236)

CD3 zeta (phospho Y83) Western blot staining using rabbit Anti-CD3 zeta (phospho Y83) antibody

Western Blotting using Recombinant Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y], ab68236. Publication image from Jorge, I. et al., 2016, Nat Commun, 27091106. Legend direct from paper.

Aurora A inhibition impairs TCR signalling pathways.(a) Immunoblottings showing phosphorylation of the indicated molecules in lysates of J77 Jurkat T cells pretreated with vehicle (DMSO) or Aurora A inhibitor (MLN8237) and conjugated for the indicated times with SEE-pulsed Raji B cells. (b) Quantification of blots as in a from four to six independent experiments. Error bars represent interquartile range. Medians were compared with a Friedman test (*P<0.05). n.s., nonsignificant. (c) Immunoblottings showing phosphorylation of the indicated molecules in lysates of DMSO- or MLN8237-pretreated primary human CD4+ T cells conjugated for the indicated times with anti-CD3/CD28-coated beads. (d) Quantification of blottings as in c from four to six independent experiments. Error bars represent interquartile range. Medians were compared with a Friedman test (*P<0.05). n.s., nonsignificant.

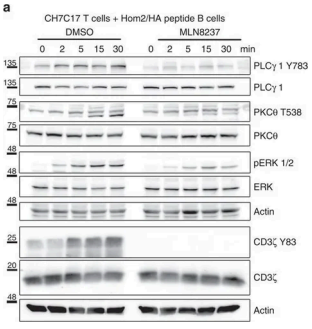


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Western blot - Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y] (ab68236)

CD3 zeta (phospho Y83) Western blot staining using rabbit Anti-CD3 zeta (phospho Y83) antibody

Western Blotting using Recombinant Anti-CD3 zeta (phospho Y83) antibody [EP776(2)Y], ab68236. Publication image from Jorge, I. et al., 2016, Nat Commun, 27091106. Legend direct from paper.

Aurora A inhibition impairs TCR signalling and gene expression.(a) Immunoblottings showing phosphorylation of the molecules indicated in lysates of CH7C17 Jurkat T cells pretreated with DMSO or MLN8237 and conjugated for the indicated times with HA-peptide-pulsed Hom2 B cells. (b) Quantification of blots as in a–c from four to six independent experiments. Error bars represent interquartile range. Medians were compared with a Friedman test (*P<0.05). n.s., nonsignificant. (c) IL2, CD69 and CD25 mRNA levels in primary human CD4+ T cells pretreated with DMSO, MLN8237 (10 µM) or the Aurora B inhibitor AZD1152 (100 nM) and activated by settling on anti-CD3/CD28-coated plates for 4 h. mRNA levels were normalized to the housekeeping gene GAPDH and the levels of the target mRNA in non-stimulated cell levels. Error bars represent interquartile rage. Medians were compared with a Mann–Whitney test. **P<0.01.

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