Product Datasheet

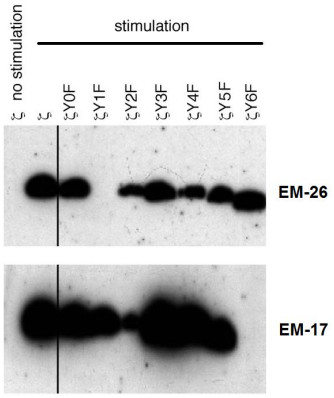
Anti-Hu CD3 zeta (pY72) Purified (orb179876)

Description	Mouse monoclonal antibody to CD3 zeta
Reactivity	Human, Mouse
Conjugation	Unconjugated
Tested Applications	FC, ICC, WB
Immunogen	A phospho specific peptide corresponding to the amino acids surrounding tyrosine 72 of mouse CD3 zeta linked to KLH
Target	CD3 zeta (pY72)
Preservatives	Phosphate buffered saline (PBS), pH 7.4, 15 mM sodium azide
Concentration	1 mg/ml
Storage	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
Note	For research use only
Application notes	Western blotting: Recommended dilution: 2 - 5 µg/ml; positive control: Jurkat cells lysate treated with pervanadate, splenocyte lysate of Balb/c or F1 mouse treated with pervanadate, non-reducing conditions recommended. Flow cytometry: Intracellular staining; recommended dilution: 1-9 µg/ml; positive control: Jurkat cells treated with pervanadate, T-cells from lymph nodes of OT-1 mouse treated with pervanadate.
Isotype	Mouse IgG2b
Clonality	Monoclonal
Clone Number	EM-26
Antibody Type	Primary Antibody

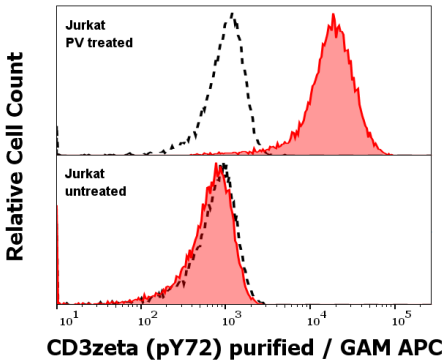
Biorbyt Ltd.
7 Signet Court, Swann's Road,
Cambridge, CB5 8LA, United Kingdom
Email: info@biorbyt.com, support@biorbyt.com
Phone: +44 (0) 1223 859-353 | Fax: +1 (415) 651-8558

Biorbyt LLC.
68 TW Alexander Drive,
Durham, NC, 27713, United States
Email: info@biorbyt.com, support@biorbyt.com
Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

Purity	Purified by protein-A affinity chromatography.
Uniprot ID	**P20963**
Entrez	**919**
Expiration Date	12 months from date of receipt.



Western blotting analysis using monoclonal antibodies EM-26 (anti-CD3 zeta phospho-Tyr72) and EM-17 (anti-CD3 zeta phospho-Tyr153) reacting with phosphorylated particular human CD3 zeta mutants. The Y1F and Y6F mutatants lack phosphotyrosine 72 and 153, respectively.



Anti-Hu CD3 zeta (p72) purified antibody (clone EM-26) works in Flow Cytometry application. Analysis of the antibody staining was performed on Jurkat cells treated or untreated with pervanadate (PV) prior to the fixation and permeabilization of cell suspension with cold methanol. Anti-Hu CD3 zeta (pY72) purified antibody (concentration in sample 1 µg/ml, red-filled histogram) binds specifically to phosphorylated tyrosine 72 (pY72) of CD3 zeta chain in PV treated, methanol permeabilized Jurkat cells (upper panel), but not to untreated methanol permeabilized control cells (lower panel). Level of non-specific binding was assessed using Mouse IgG1 isotype control PE (MOPC-21) under same conditions (concentration in sample 1 µg/ml, black-dashed histogram).

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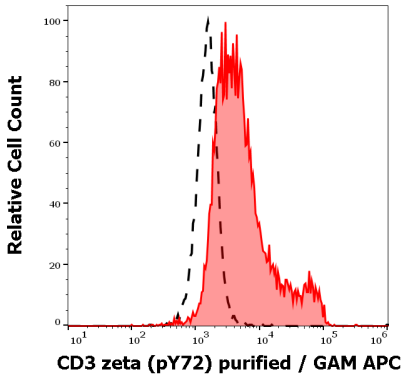
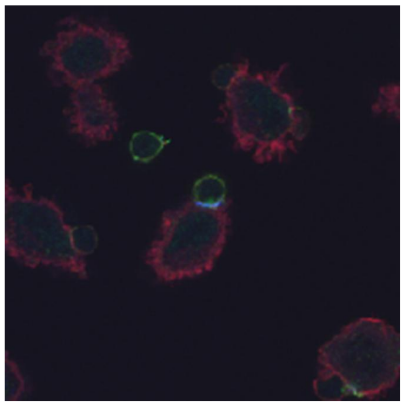
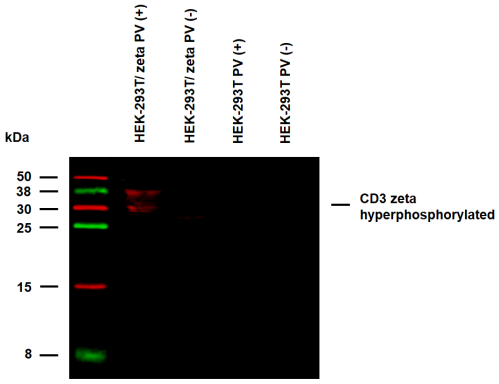


Separation of pervanadate stimulated Jurkat cell suspension stained using anti-human CD3 zeta (pY72) (EM-26) purified antibody (concentration in sample 3 µg/ml, GAM APC, red-filled) from pervanadate stimulated Jurkat cell suspension unstained by primary antibody (GAM APC, black-dashed) in flow cytometry analysis (intracellular staining).



Immunocytochemistry detection of phosphorylated CD3 zeta (EM-26; light blue) in immunological synapse formed between the lymph node naïve T cells from AND TCR transgenic mice and DCEK cells loaded with MCC peptide, after 20 min. Total CD3 zeta indicated in green, actin cytoskeleton in red.



Anti-Hu CD3 zeta (pY72) Purified (clone EM-26) specificity verification by WB. The specificity of EM-26 antibody to phosphorylated Tyr 72 (CD3 zeta chain) was assessed by analysis of binding signals in HEK293T transfected with CD3 zeta/ZAP-70 construct followed by pervanadate (PV) treatment in comparison to the series of control cells - PV untreated transfectants, and both PV treated and untreated mock HEK293T cells. Western blotting analysis was performed on whole cell extracts (RIPA lysis buffer with PhosSTOP and pervanadate), mixed and heated (100°C, 5 min) with non-reducing SDS-loading buffer. Samples were resolved using 15% Tris-glycine SDS gel electrophoresis. Nitrocellulose membrane blot was probed with mouse IgG2b monoclonal antibody EM-26 (1 µg/ml). Subclass-specific secondary antibody IRDye 680LT Goat-anti-Mouse IgG (red) was used for fluorescent Western blot detection.

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