

Datasheet for ABIN2749058
anti-CD247 antibody (pTyr142)[Go to Product page](#)

3 Images

Overview

Quantity:	0.1 mg
Target:	CD247
Binding Specificity:	pTyr142
Reactivity:	Human, Mouse
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This CD247 antibody is un-conjugated
Application:	Western Blotting (WB), Flow Cytometry (FACS)

Product Details

Immunogen:	CP1 (pTyr142) - KLH
Clone:	EM-54
Isotype:	IgG1
Specificity:	The mouse monoclonal antibody EM-54 recognizes phosphorylated intracellular tyrosine 142 of CD3 zeta chain (CD247), which is a component of TCR/CD3 complex expressed on T cells.
Cross-Reactivity (Details):	Human, Mouse
Purification:	Purified by protein-A affinity chromatography.
Purity:	> 95 % (by SDS-PAGE)

Target Details

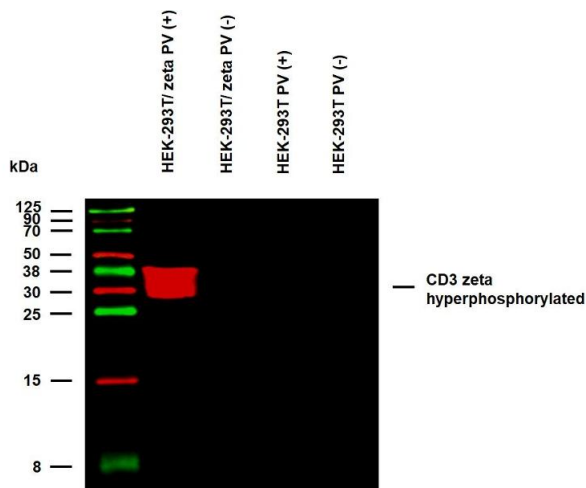
Target:	CD247
Alternative Name:	CD3 zeta (CD247 Products)
Background:	CD247 Molecule,CD3 complex is crucial in transducing antigen-recognition signals into the cytoplasm of T cells and in regulating the cell surface expression of the TCR complex. T cell activation through the antigen receptor (TCR) involves the cytoplasmic tails of the CD3 subunits CD3 gamma, CD3 delta, CD3 epsilon and CD3 zeta (CD247). These CD3 subunits are structurally related members of the immunoglobulins super family encoded by closely linked genes on human chromosome 11. The CD3 components have long cytoplasmic tails that associate with cytoplasmic signal transduction molecules. This association is mediated at least in part by a double tyrosine-based motif present in a single copy in the CD3 subunits. CD3 may play a role in TCR-induced growth arrest, cell survival and proliferation.,CD3 zeta chain, CD247, T3Z, TCRz, IMD25
Gene ID:	919
UniProt:	P20963
Pathways:	TCR Signaling , CXCR4-mediated Signaling Events , Ubiquitin Proteasome Pathway

Application Details

Application Notes:	Flow cytometry: Recommended dilution: 1-4 µg/mL. Intracellular staining.
Restrictions:	For Research Use only

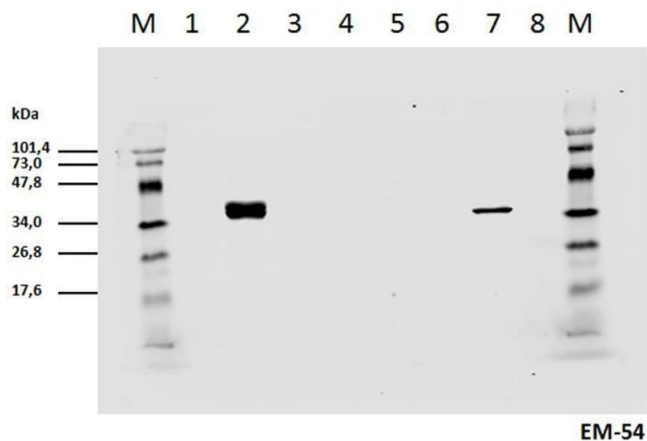
Handling

Concentration:	1 mg/mL
Buffer:	Phosphate buffered saline (PBS), pH 7.4, 15 mM sodium azide
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	4 °C
Storage Comment:	Store at 2-8°C. Do not freeze.



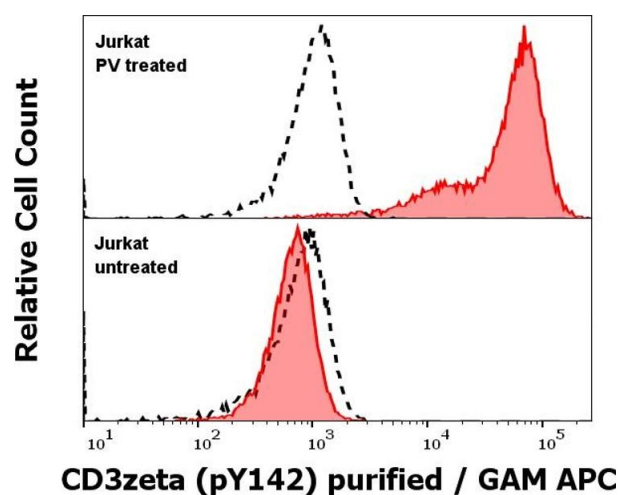
Western Blotting

Image 1. Anti-Hu CD3 zeta (pY142) Purified (clone EM-54) specificity verification by WB. The specificity of EM-54 antibody to phosphorylated Tyr 142 (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 IgG1 monoclonal antibody EM-54 (1 µg/mL). Subclass-specific secondary antibody IRDye 680LT Goat-anti-Mouse IgG (red) was used for fluorescent Western blot detection.



Western Blotting

Image 2. Cell lysates of HEK293T/17 cells transiently transfected with expression vectors harboring genes for mCD3zeta wild type and six different mCD3zeta mutants, where particular ITAM tyrosines were substituted with phenylalanines, were prepared. Subsequently the lysates (non-reducing conditions) were immunoblotted with mouse mAb anti-pY142 mCD3zeta (clone EM-54). 1: Wt mCD3zeta pervanadate non-stimulated 2: Wt mCD3zeta pervanadate stimulated 3: mut. mCD3zeta Y/F2-6 4: mut. mCD3zeta Y/F1 and 3-6 5: mut. mCD3zeta Y/F1-2 and 4-6 6: mut. mCD3zeta Y/F1-3 and 5-6 7: mut. mCD3zeta Y/F1-4 and 6 (thus only pY142 remains native) 8: mut. mCD3zeta Y/F1-5



Flow Cytometry

Image 3. Anti-Hu CD3 zeta (pY142) purified antibody (clone EM-54) 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 (pY142) purified antibody (concentration in sample 1 $\mu\text{g/mL}$, red-filled histogram) binds specifically to phosphorylated tyrosine 142 (pY142) 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 $\mu\text{g/mL}$, black-dashed histogram).