

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human DR3/TNFRSF25 in direct ELISAs and Western blots. Shows 100% cross-reactivity with recombinant mouse (rm) DR3/TNFRSF25 and no cross-reactivity with recombinant human (rh) 4-1BB, rhCD27, rhCD30, rhCD40, rhDR6, rhBAFF R, rhFas, rhGITR, rhLTR β , rhNGF R, rhOPG, rmOX40, rhRANK, rhTAJ, rhTNF RI, rhTNF RII, or rhHVEM.
Source	Monoclonal Mouse IgG ₁ Clone # 59204
Purification	Protein A or G purified from ascites
Immunogen	Mouse myeloma cell line NS0-derived recombinant human DR3 isoform 1 Gln25-Phe201 Accession # Q93038
Formulation	Lyophilized from a 0.2 μ m filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 μ m filtered solution in PBS.

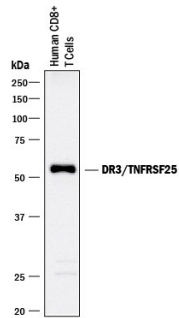
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	2 μ g/mL	See Below
Flow Cytometry	0.25 μ g/10 ⁶ cells	See Below
CytoF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

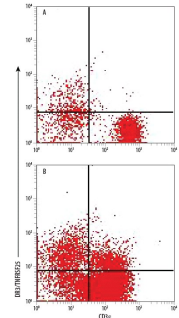
DATA

Western Blot



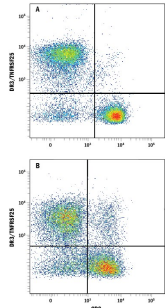
Detection of Human DR3/TNFRSF25 by Western Blot. Western blot shows lysates of human CD8⁺ T cells. PVDF membrane was probed with 2 μ g/mL of Mouse Anti-Human/Mouse DR3/TNFRSF25 Monoclonal Antibody (Catalog # MAB943) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for DR3/TNFRSF25 at approximately 55 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



Detection of DR3/TNFRSF25 in Human PBMCs by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) either (A) untreated or (B) treated with PMA and Calcium Ionomycin for 24 hours were stained with Mouse Anti-Human DR3/TNFRSF25 Monoclonal Antibody (Catalog # MAB943) followed by Phycoerythrin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0102B) and Mouse Anti-Human CD3 ϵ APC-conjugated Monoclonal Antibody (Catalog # FAB100A). Quadrant markers were set based on control antibody staining (Catalog # MAB002).

Flow Cytometry



Detection of DR3/TNFRSF25 in Mouse Splenocytes by Flow Cytometry. Mouse splenocytes either (A) untreated or (B) treated with PMA and Calcium Ionomycin for 24 hours were stained with Mouse Anti-Human DR3/TNFRSF25 Monoclonal Antibody (Catalog # MAB943) followed by Allophycocyanin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0101B) and Rat Anti-Mouse CD3 PE-conjugated Monoclonal Antibody (Catalog # FAB4841P). Quadrant markers were set based on control antibody staining (Catalog # MAB002).

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Death receptor 3 (DR3), also known as lymphocyte-associated receptor of death (LARD), WSL-1, APO3, TRAMP and TR3, is a glycoprotein belonging to the TNF receptor superfamily (TNFRSF) (1 - 5). DR3 was formerly designated TNFRSF12 when it was thought to be a receptor for TWEAK/TNFRSF12 (6). However, work disavowed the DR3:TWEAK interaction and DR3 is now designated TNFRSF 25 (7). By alternative splicing, at least 11 distinct human DR3 transcripts encoding secreted or type I membrane proteins exist (7). The human DR3 isoform 1 cDNA encodes a 417 amino acid residue (aa) transmembrane precursor with a 24 aa signal peptide, a 175 aa extracellular domain containing four cysteine-rich repeats and two potential N-glycosylation sites, a 21 aa transmembrane region and a 195 aa cytoplasmic region with one death domain. DR3 is one of six within the TNF R superfamily that contains a death domain in its cytoplasmic region. It is most closely related to TNF R1 and FAS/CD95, sharing 29% and 23% aa sequence identity, respectively. DR3 is expressed primarily in tissues enriched in lymphocytes. Whereas naïve B and T cells express multiple truncated DR3 isoforms but not the transmembrane isoform 1, upon T cell activation, expression of the transmembrane DR3 isoform 1 predominates. TL1A/VEGI, a TNF superfamily ligand, has been shown to bind and activate DR3 (8). Depending on the cell context, ligation of DR3 by TL1A can trigger one of two signaling pathways. On primary T cells, TL1A induces NF-kappa-B activation and a costimulatory signal to increase IL-2 responsiveness and the secretion of proinflammatory cytokines. However, in a tumor cell line, TF-1, TL1A has been shown to induce caspase activity and apoptosis. In DR3-null mice, an impairment of negative selection and anti-CD3-mediated thymocyte apoptosis is observed.

References:

1. Chinnaiyan, A.M. *et al.* (1996) *Science* **274**:990.
2. Kitson, J. *et al.* (1996) *Nature* **384**:372.
3. Bodmer, J-L. *et al.* (1997) *Immunity* **6**:79.
4. Screatton, G.R. *et al.* (1997) *Proc. Nat. Acad. Sci. USA* **94**:4615.
5. Marsters, S.A. *et al.* (1996) *Curr. Biol.* **6**:1669.
6. Marsters, S.A. *et al.* (1998) *Curr. Biol.* **8**:525.
7. Kaptein, A. *et al.* (2000) *FEBS Lett.* **485**:135.
8. Migone, T-S. *et al.* (2002) *Immunity* **16**:479.