RD SYSTEMS a biotechne brand

Monoclonal Mouse IgG₁ Clone # 6402 Catalog Number: MAB10564

DESCRIPTION	
Species Reactivity	Human
Specificity	Detects human TNF-α in direct ELISAs.
Source	Monoclonal Mouse IgG ₁ Clone # 6402
Purification	Protein A or G purified from cell culture supernatant
Immunogen	<i>E. coli-</i> derived recombinant human TNF-α Gly57-Leu233 Accession # P01375
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either Iyophilized or as a 0.2 μm filtered solution in PBS.

APPLICATIONS

DATA

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			
Intracellular Staining by Flow Cytometry	0.25 µg/10 ⁶ cells	Human peripheral blood mononuclear cells (PBMCs) treated with PMA and Ca2+ionomycin fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005)			
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.				

Intracellular Staining by Flow Cytometry

TNF-cc	u ⁶ A u ⁴ u ⁸ u ²		
Mouse IgG ₁		23 ³ 23 ⁴ 32 23 ³ 23 ⁴ 32 23 ³ 23 ⁴ 32 23 ³ 23 ⁴ 32	5

Detection of TNF- alpha in Human PBMCs by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) treated with PMA and Ca2+ionomycin for 3.5 hours were stained with (A) Mouse Anti-Human TNF-alpha Monoclonal Antibody (Catalog # MAB10564) or (B) isotype control antibody (Catalog # MAB002) followed by Anti-Mouse IgG PE-conjugated Secondary Antibody (Catalog # F0102B) and Mouse anti-Human CD3 APC-conjugated Monoclonal Antibody (Catalog # FAB100A). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005). Staining was performed using our Staining Intracellular Molecules protocol.

PREPARATION AND S	TORAGE	
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	 Use a manual defrost freezer and avoid repeated freeze-thaw cycles. 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

Tumor necrosis factor alpha (TNF-alpha, TNF- α, TNFA), also known as Cachectin and TNFSF2, is the prototypic ligand of the TNF superfamily. It is a pleiotropic molecule that plays a central role in inflammation, immune system development, apoptosis, and lipid metabolism. TNF- is produced by several lymphoid cells as well as by astrocytes, endothelial cells, and smooth muscle cells. Human TNF-alpha consisits of a 35 amino acid (aa) cytoplasmic domain, a 21 aa transmembrane segment, and a 177 aa extracellular domain (ECD). Within the ECD, human TNF-alpha shares 97% aa sequence identity with rhesus and 71%-92% with bovine, canine, cotton rat, equine, feline, mouse, porcine, and rat TNF-alpha. TNF-alpha is produced by a wide variety of immune, epithelial, endothelial, and tumor cells. TNF-alpha is assembled intracellularly to form a noncovalently linked homotrimer which is expressed on the cell surface. Cell surface TNF-alpha can induce the lysis of neighboring tumor cells and virus infected cells, and it can generate its own downstream cell signaling following ligation by soluble TNFR I. Shedding of membrane bound TNF-alpha by TACE/ADAM17 releases the bioactive cytokine, a 55 kDa molecular weight soluble trimer of the TNF-alpha extracellular domain. TNF-alpha binds the ubiquitous 55-60 kDa TNF RI and the hematopoietic cell-restricted 80 kDa TNF RII, both of which are also expressed as homotrimers present on virtually all cell types. Both type I and type I receptors bind TNF-alpha with comparable affinity, although only TNF RI contains a cytoplasmic death domain which triggers the activation of apoptosis. Soluble forms of both types of receptors are released and can neutralize the biological activity of TNF-alpha.

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