# **Technical Data Sheet**

# PE Rat Anti-Human CD120b

#### Product Information

Material Number:	552418
Alternate Name:	TNF Receptor Type II
Size:	100 tests
Vol. per Test:	20 µl
Clone:	hTNFR-M1
Immunogen:	COS-expressed recombinant human TNFRII
Isotype:	Rat IgG2b, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

#### Description

The hTNFR-M1 antibody reacts with the extracellular domain of the 75 kDa transmembrane receptor for the human cytokines, tumor necrosis factor (TNF or TNF- $\alpha$ ) and lymphotoxin-alpha (LT- $\alpha$ 3, aka, lymphotoxin or TNF- $\beta$ ). This receptor is referred to as the p75 or Type II Tumor Necrosis Factor Receptor (TNFRII) [aka, CD120b]. Human TNFRII proteins are expressed by hematopoietic cells including macrophages, neutrophils, lymphocytes, thymocytes and mast cells. TNFRII is expressed by a variety of other cell types including endothelial cells, cardiac myocytes and prostate cells. Naive B cells express very low or undetectable levels of TNFRII whereas mature erythrocytes and platelets are uniformly negative for TNFRII expression. The immunogen used to generate the hTNFR-M1 hybridoma was COS- expressed recombinant human TNFRII.



Expression of cell surface TNFRII by lysed whole human blood. Whole human blood was blocked with normal polyclonal human IgG, stained with R-PE-conjugated hTNFR-M1 ( $20 \mu/1X10^{6}$  cells, Cat No. 552418) and subsequently lysed with BD Pharm Lyse<sup>TM</sup> (Cat No. 555899). Staining with the hTNFR-M1 antibody (filled histogram) is compared to staining obtained using the isotype control antibody, PE anti-Rat IgG2b  $\kappa$ , isotype control (Cat. No. 553989; open histogram). The histograms in the figure were derived from gated events with the light scattering characteristics of viable lymphocytes (left panel), monocytes (center panel) and granulocytes (right panel). Note: Certain human cell lines or cell types (e.g., neutrophils, monocytes) can first be treated with reagents that block receptors for the Fc regions of immunoglobulin to avoid nonspecific immunofluorescent staining mediated by Fc receptors.

### **Preparation and Storage**

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze. The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

#### **Application Notes**

A	Application						
	Flow cytometry	Routinely Tested					

#### **Recommended Assay Procedure:**

Immunofluorescent Staining and Flow Cytometric Analysis: The R-PE-conjugated hTNFR-M1 (Cat. No. 552418) antibody can be used for the immunofluorescent staining ( $20 \ \mu l / 1X10^{\circ}6$  cells) and flow cytometric analysis of human nucleated cells to measure their expressed levels of surface TNFRII. An appropriate immunoglobulin isotype control is clone A95-1 (Cat. No. 553989). Please note also that as a consequence of in vivo or in vitro activation, cell surface TNFRII can either be shed by cells or transiently expressed at higher levels. As a result, cellular activation can affect the overall expressed level of surface TNFRII.

#### **BD Biosciences**

bdbiosciences.com									
United States 877.232.8995	<b>Canada</b> 888.268.5430	Europe 32.53.720.550	<b>Japan</b> 0120.8555.90	Asia Pacific 65.6861.0633	Latin America/Caribbear 0800.771.7157				
For country-specific contact information, visit bdbiosciences.com/how to order/									
Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton Dickinson and Company is strictly prohibited. For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale. BD, BD Logo and all other trademarks are the property of Becton, Dickinson and Company. ©2011 BD									

## **Suggested Companion Products**

Catalog Number	Name	Size	Clone
555899	Lysing Buffer	100 ml	(none)
553989	PE Rat IgG2b, κ Isotype Control	0.1 mg	A95-1
554656	Stain Buffer (FBS)	500 ml	(none)

#### Product Notices

- 1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^{6}$  cells in a 100-µl experimental sample (a test).
- 2. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
- 3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before
- discarding to avoid accumulation of potentially explosive deposits in plumbing.
- 4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- 5. An isotype control should be used at the same concentration as the antibody of interest.
- 6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

#### References

Aggarwal S, Gollapudi S, Gupta S. Increased TNF-alpha-induced apoptosis in lymphocytes from aged humans: changes in TNF-alpha receptor expression and activation of caspases. *J Immunol.* 1999; 162(4):2154-2161. (Biology)

Brockhaus M, Schoenfeld HJ, Schlaeger EJ, Hunziker W, Lesslauer W, Loetscher H. Identification of two types of tumor necrosis factor receptors on human cell lines by monoclonal antibodies. *Proc Natl Acad Sci U S A*. 1990; 87(8):3127-3131. (Biology)

Browning JL, Dougas I, Ngam-ek A, et al. Characterization of surface lymphotoxin forms. Use of specific monoclonal antibodies and soluble receptors. *J Immunol.* 1995; 154(1):33-46. (Clone-specific: Flow cytometry)

Erikstein BK, Smeland EB, Blomhoff HK, et al. Independent regulation of 55-kDa and 75-kDa tumor necrosis factor receptors during activation of human peripheral blood B lymphocytes. *Eur J Immunol.* 1991; 21(4):1033-1037. (Biology)

Gehr G, Gentz R, Brockhaus M, Loetscher H, Lesslauer W. Both tumor necrosis factor receptor types mediate proliferative signals in human mononuclear cell activation. *J Immunol.* 1992; 149(3):911-917. (Biology)

Heilig B, Mapara M, Brockhaus M, Krauth K, Dörken B. Two types of TNF receptors are expressed on human normal and malignant B lymphocytes. Clin Immunol Immunopathol. 1991; 61(2):260-267. (Clone-specific: Flow cytometry)

Hohmann HP, Remy R, Brockhaus M, van Loon AP. Two different cell types have different major receptors for human tumor necrosis factor (TNF alpha). J Biol Chem. 1989; 264(25):14927-14934. (Biology)

Munker R, DiPersio J, Koeffler HP. Tumor necrosis factor: receptors on hematopoietic cells. *Blood.* 1987; 70(6):1730-1734. (Clone-specific: Flow cytometry) Wallach D, Engelmann H, Nophar Y, et al. Soluble and cell surface receptors for tumor necrosis factor. *Agents Actions Suppl.* 1991; 35:51-57. (Biology) Ware CF, Crowe PD, Vanarsdale TL, et al. Tumor necrosis factor (TNF) receptor expression in T lymphocytes. Differential regulation of the type I TNF receptor during activation of resting and effector T cells. *J Immunol.* 1991; 147(12):4229-4238. (Biology)