

## Product Information

### Monoclonal Anti-Tumor Necrosis Factor Receptor II clone 22221

produced in mouse, purified immunoglobulin

Catalog Number **T1815**

#### Product Description

Monoclonal Anti-Tumor Necrosis Factor Receptor II (TNF RII; mouse IgG2a isotype) is derived from the 22221 hybridoma produced by the fusion of mouse myeloma with B cells obtained from a mouse immunized with recombinant human TNF-RII, extracellular domain, expressed in *E. coli*. The antibody is purified from ascites fluid using protein A affinity chromatography.

Monoclonal Anti-Tumor Necrosis Factor Receptor II may be used to neutralize the biological activity of rhTNF RII, but not rhTNF RI. The antibody will also detect human TNF RII by immunocytochemistry.

TNF RII (p75, CD120b) is a 75 kDa transmembrane glycoprotein originally isolated from a human lung fibroblast library.<sup>1</sup> Among the multitude of cells known to express TNFRII are monocytes,<sup>2</sup> endothelial cells,<sup>3</sup> Langerhans cells,<sup>4</sup> and macrophages.<sup>5</sup>

Mouse to human amino acid sequence identity in the TNF RII cytoplasmic domain is 73%, while amino acid sequence identity in the extracellular region falls to 58%.<sup>6</sup> This drop in extracellular identity is reflected in the observation that human TNF- $\alpha$  is not active in the mouse system.<sup>6</sup> TNF RII to TNF RI amino acid sequence identity is only about 20% in the extracellular region and 5% in the cytoplasmic domain.<sup>6</sup> TNF RII consists of a 240 amino acid residue extracellular region, a 27 amino acid residue transmembrane segment, and a 173 amino acid residue cytoplasmic domain.<sup>7,8</sup>

TNF RI and TNF RII are members of a family of structurally related membrane receptors that includes lymphotoxin receptor, Fas, WSL-1, DR4, CD40, CD30, CD27, 4-1BB, OX40, and p75 nerve growth factor receptor.<sup>9</sup> Members of the TNFR family can interact through their cytoplasmic domains with a range of intracellular signalling proteins, most of which fall into two distinct groups.

The first group is the death domain-containing proteins, including TRADD, FADD/MORT1, and RIP, which associate directly with receptors also containing death domains, such as TNF RI and Fas.<sup>10-12</sup> The second group is the TRAF proteins. TRAF1 and TRAF2 were originally identified by their association with the cytoplasmic domain of TNFR2.<sup>13</sup> TRAF proteins appear to function as adaptor proteins. TRAF2 directly binds to at least eight intracellular molecules, including TRAF1, c-IAP1, c-IAP2, I-TRAF/TANK, A20, TRIP, RIP, and NIK.<sup>13-20</sup> The best characterized TRAF-mediated signal transduction pathway is the activation of NF-B transcription factors. TRAF2 mediates NF-B activation via the recruitment of the serine/threonine kinase NIK,<sup>20</sup> which can in turn activate CHUK, an IB-specific kinase that triggers IB degradation.<sup>21,22</sup> In addition to recruiting mediators of NF-B activation, TRAF2 can bind at least three other molecules (I-TRAF/TANK, A20, TRIP) that inhibit its ability to activate NF-B.<sup>16-18</sup>

#### Reagent

Lyophilized from 0.2  $\mu$ m-filtered solution in phosphate buffered saline containing carbohydrates.

#### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

#### Preparation Instructions

To one vial of lyophilized powder, add 1 mL of 0.2  $\mu$ m-filtered phosphate buffered saline to produce a 0.5 mg/mL stock solution of antibody. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

### Storage/Stability

Prior to reconstitution, store at  $-20\text{ }^{\circ}\text{C}$ . Reconstituted product may be stored at  $2-8\text{ }^{\circ}\text{C}$  for up to one month. For prolonged storage, freeze in working aliquots at  $-20\text{ }^{\circ}\text{C}$ . Avoid repeated freezing and thawing.

### Product Profile

**Neutralization:** The antibody was tested for its ability to neutralize the biological effect of TNF RII in a cytolytic assay using mouse L929 cells, in the presence of TNF- $\alpha$ .<sup>23</sup> The ND<sub>50</sub> of the antibody is defined as the concentration of antibody resulting in a one-half maximal inhibition of the soluble receptor activity on a responsive cell line, when the receptor is present at a concentration just high enough to elicit a maximum response. The exact concentration of antibody required to neutralize rhTNF RII activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

**Immunocytochemistry:** a working concentration of 8-25  $\mu\text{g}/\text{mL}$  is recommended using immersion fixed human peripheral blood lymphocytes.

**Note:** In order to obtain the best results in various techniques and preparations, determination of optimal working dilutions by titration test is recommended.

### References

1. Smith, C.A., et al., *Science*, **248**, 1019 (1990).
2. Lien, E., et al., *Eur. J. Immunol.*, **25**, 2714 (1995).
3. Bradley, J.R., et al., *Am. J. Pathol.*, **146**, 27 (1995).
4. Wang, B., et al., *Immunology*, **88**, 284 (1996).
5. de Rochemonteix, B.G., et al., *Am. J. Respir. Cell Mol. Biol.*, **14**, 279 (1996).
6. Lewis, M., et al., *Proc. Natl. Acad. Sci. USA*, **88**, 2830 (1991).
7. Gruss, H-J., and Dower, S.K., *Blood*, **85**, 3378 (1995).
8. Smith, C.A., et al., *Science*, **248**, 1019 (1990).
9. Smith, C. A., et al., *Cell*, **76**, 959 (1994).
10. Hsu, H., et al., *Cell*, **81**, 495 (1995).
11. Chinnaiyan, A.M., et al., *Cell*, **81**, 505 (1995).
12. Boldin, M.P., et al., *J. Biol. Chem.*, **270**, 7795 (1995).
13. Rothe, M., et al. *Cell*, **78**, 681 (1994).
14. Cheng, G., and Baltimore, D., *Genes Dev.*, **10**, 963 (1996).
15. Rothe, M., et al., *Cell*, **83**, 1243 (1995).
16. Rothe, M., et al., *Proc. Natl. Acad. Sci. USA*, **93**, 8241 (1996).
17. Song, H.Y., et al., *Proc. Natl. Acad. Sci. USA*, **93**, 6721 (1996).
18. Lee, S.Y., et al., *J. Exp. Med.*, **185**, 1275 (1997).
19. Hsu, H., et al., *Immunity*, **4**, **387** (1996).
20. Malinin, N.L., et al., *Nature*, **385**, 540 (1997).
21. Régnier, C.H., et al., *Cell*, **90**, 373 (1997).
22. DiDonato, J.A., et al., *Nature*, **388**, 548 (1997).
23. Matthews, N., and Neale, M.L., in "Lymphokines and Interferons, A Practical Approach", Clemens, M.J., et al., (Eds.), IRL Press, p. 296 (1987).

KCP,PHC,TMS 06/16-1