



## PRODUCT DATA SHEET

### **Product: TNF- $\alpha$ , soluble (recombinant human) Kit**

**Cat. No.:** TN-011

#### **Kit Contents:**

- 50  $\mu$ g lyophilized recombinant human soluble TNF- $\alpha$  plus PBS salts (1 mg/mL after reconstitution)
- 2 x 50  $\mu$ g lyophilized Enhancer for Ligands (anti-FLAG antibody) plus PBS salts (1 mg/mL after reconstitution)

#### **Principle:**

Tumor Necrosis factor-alpha (TNF- $\alpha$ ) is a 35 kDa type II transmembrane protein that is produced by many cell types including neutrophils, activated lymphocytes, macrophages, endothelial cells, and smooth muscle cells. TNF- $\alpha$  interacts with two different receptors, designated TNF-R1 and TNF-R2. Binding of TNF- $\alpha$  to either receptor leads to activation of NF- $\kappa$ B and JNK. Binding of TNF- $\alpha$  to TNF-R1 also induce apoptosis. Membrane bound TNF- $\alpha$  is efficiently cleaved from the cell surface by a metalloprotease. The soluble form of TNF- $\alpha$  binds to and activates TNF-R1, while activation of TNF-R2 requires membrane bound (or cross-linked) TNF- $\alpha$ .

#### **Specificity:**

Recombinant human soluble TNF- $\alpha$  recognizes human and mouse TNF-R1 and TNF-R2. In the presence of cross-linking enhancer, rhsTNF- $\alpha$  preferentially binds to human and mouse TNF-R2.

#### **Species Reactivity:**

Human and mouse. Other species not tested.

#### **Applications:**

The extracellular domain of the rhsTNF- $\alpha$  (aa 85-233) is fused at the N-terminus to FLAG-tag and an 8 aa linker peptide for induction of TNF-R1 and TNF-R2 mediated responses. rhsTNF- $\alpha$  binds to TNF-R1 and triggers various biological effects, including apoptosis in sensitive cell lines. In the presence of the secondary cross-linking antibody, rhsTNF- $\alpha$  also activates TNF-R2 mediated responses (human only).

#### **Protocols:**

Prepare sterile stock solutions of rhsTNF- $\alpha$  (1 mg/mL) and enhancer (1 mg/mL) by dissolving the contents of each tube in 50  $\mu$ L of sterile H<sub>2</sub>O. Further dilutions should be made with medium containing 5% FCS. To induce TNF-R2 mediated responses, incubate cells in the presence of rhsTNF- $\alpha$  and 2  $\mu$ g/mL enhancer. The amount required will depend on experimental conditions; it will be necessary to perform a titration to determine optimal concentrations.

Recombinant, human sTNF- $\alpha$  exerts its biological activity in a concentration range of 0.1 – 1 ng/mL on WEHI 164 cells. Concentrations of rhsTNF- $\alpha$  required to kill cells may vary depending on cell type studied. Cell viability was determined using a MTT-based cell proliferation assay kit.

#### **Storage:**

Store at -20°C. Aliquot solutions to avoid repeated freeze/thaw. Once rehydrated it is recommended to prepare appropriate aliquots and to store them at -20°C. Avoid repeated freezing and thawing; activity may decrease.

#### **Limitations:**

For *in vitro* research use only. Not for use in diagnostics or in humans.

#### **Warranty:**

No warranties, expressed or implied, are made regarding the use of this product. **KAMIYA BIOMEDICAL COMPANY** is not liable for any damage, personal injury, or economic loss caused by this product.