

# Rat TNF- $\alpha$ ELISA Development Kit

(Catalog #K1052-1000; 10 x 96 plates; Store kit at  $-20^{\circ}\text{C}$ )

## I. Description:

Rat TNF- $\alpha$  ELISA development kit contains the key components required for the quantitative measurement of natural and/or recombinant TNF- $\alpha$  in a sandwich ELISA format within the range of 32-3000 pg/ml. Using the ELISA protocol described below, the recommended microplates, reagents and solutions, the components supplied in this kit are sufficient to assay rTNF- $\alpha$  in approximately 1000 ELISA plate wells.

## II. Kit Components:

**Capture Antibody:** 100  $\mu\text{g}$  of rabbit anti-rTNF- $\alpha$ . Reconstitute in 1-ml sterile water for a concentration of 100  $\mu\text{g}/\text{ml}$ .

**Detection Antibody:** 50  $\mu\text{g}$  of biotinylated antigen-affinity purified anti-rTNF- $\alpha$ . Reconstitute in 0.5-ml sterile water for a concentration of 100  $\mu\text{g}/\text{ml}$ .

**Rat TNF- $\alpha$  Standard:** 1  $\mu\text{g}$  of recombinant rTNF- $\alpha$ . Reconstitute in 1-ml sterile water for a concentration of 1  $\mu\text{g}/\text{ml}$ .

**Avidin Peroxidase Conjugate:** 60  $\mu\text{l}$  vial.

## III. Storage Conditions:

The reconstituted Capture Antibody and Detection Antibody can be stored at  $4^{\circ}\text{C}$  for up to 2 months or aliquoted and stored frozen at  $\leq -20^{\circ}\text{C}$  for up to 6 months.

Reconstituted hFGF-2 Standard can be kept at  $4^{\circ}\text{C}$  for 1 week, or aliquoted and stored frozen at  $\leq -20^{\circ}\text{C}$  for up to 2 months.

Upon receipt, Avidin Peroxidase Conjugate should be aliquoted into ten 6  $\mu\text{l}$  vials and stored at  $\leq -20^{\circ}\text{C}$  for up to 2 years.

## IV. Recommended Materials:

ELISA microplates (NUNC Maxisorp, Cat.# 4420404);

Tween-20 (BioVision, Cat.# 2109-100);

BSA (BioVision, Cat.# 2119-10);

ABTS Liquid Substrate Solution (Sigma, Cat.# A3219);

PBS (BioVision, Cat.# 2113-500).

### Recommended Solutions:

Wash Buffer: 0.05% Tween-20 in PBS

Block Buffer: 1% BSA in PBS

Diluent: 0.05% Tween-20, 0.1% BSA in PBS

Substrate Solution: ABTS Liquid substrate solution

## V. Plate Preparation:

1. Dilute Capture antibody with PBS to a concentration of 1  $\mu\text{g}/\text{ml}$ . Immediately add 100  $\mu\text{l}$  to each ELISA plate well. Seal the plate and incubate overnight at room temperature.
2. Aspirate the wells to remove liquid and wash plates 4 times using 300  $\mu\text{l}$  of wash buffer per well. After the last wash invert plate to remove residual buffer and blot on paper towel.
3. Add 300- $\mu\text{l}$  block buffer to each well. Incubate for at least 1 hour at room temperature.
4. Aspirate and wash plate 4 times.

## VI. ELISA Protocol:

**Standard/Sample:** Dilute standard from 3 ng/ml to zero in diluent. Add 100  $\mu\text{l}$  of standard or sample to each well in triplicate. Incubate at room temperature for at least 2 hours.

**Detection:** Wash plate 4 times. Dilute detection antibody in diluent to a concentration of 0.5  $\mu\text{g}/\text{ml}$ . Immediately add 100  $\mu\text{l}$  per well. Incubate at room temperature for 2 hours.

**Avidin Peroxidase:** Aspirate and wash plate 4 times. Dilute one 6  $\mu\text{l}$  aliquot of Avidin Peroxidase 1:2000 in diluent for total volume of 12 ml. Add 100  $\mu\text{l}$  per well. Incubate 30 minutes at room temperature.

**ABTS Liquid Substrate** (*ABTS Substrate should be at ambient temperature prior to use*): Aspirate and wash plate 4 times. Add 100- $\mu\text{l}$  substrate solution to each well. Incubate at room temperature for color development. Monitor color development with an ELISA plate reader at 405 nm with wavelength correction set at 650 nm.

**Note:** Reliable standard curves are obtained when O.D. readings do not exceed 0.2 units for the zero standard concentrations, and 1.2 units for the highest standard concentration. The plate should be monitored at 5 minutes intervals for approximately 30 minutes.

## VII. Other Featured Products:

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- Annexin V Kits & Bulk Reagents
- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- Apoptosis Inducers & Inhibitors

### Cell Fractionation System

- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
- FractionPREP Fractionation System

### Cell Proliferation & Senescence

- Quick Cell Proliferation Assay Kit
- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit

### Cell Damage & Repair

- HDAC & HAT Activity Assays & Drug Discovery Kits
- DNA Damage Quantification Kit
- Glutathione & Nitric Oxide Assay Kits

### Signal Transduction

- cAMP & cGMP Assay Kits
- Akt & JNK Activity Assay Kits
- Beta-Secretase Activity Assay Kit

### Adipocyte and Lipid Transfer

- Human/Mouse/Rat Adiponectins, Survivin, and Leptin
- CETP Activity Assay & Drug Discovery Kits
- Cholesterol Quantitation Kit

### Growth Factors, Cytokines, and Chemokines