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Data Sheet

Human IL-6 Standard TMB ELISA Development Kit

Kit Catalog# 900-T16K (900-T16 + HRP Conjugate) **Range of Detection: 16-2000 pg/ml**

Lot# 0519T016

DESCRIPTION

Contains the key components required for quantitative measurement of natural and/or recombinant Human IL-6 in a sandwich ELISA format within the range of 16-2000 pg/ml. Includes specially formulated standard of Recombinant Human IL-6 (Catalog# AF-200-06). Components supplied in this kit are sufficient to assay approximately 1000 ELISA plate wells using the protocol below, the recommended microplates, reagents, and solutions.

RECONSTITUTION & STORAGE

Catalog# 900-T16 Components:

- **Capture Antibody*:** 51µg of Goat Anti-Human IL-6 + 0.5mg D-mannitol. Centrifuge vial prior to opening. Reconstitute in 0.51ml sterile water for a concentration of 100µg/ml.
- **Detection Antibody*:** 14µg of Biotinylated Rabbit Anti-Human IL-6 + 1.4mg BSA. Centrifuge vial prior to opening. Reconstitute in 0.14ml sterile water for a concentration of 100µg/ml.
- **Standard*:** 1µg of Recombinant Human IL-6 + 2.2mg BSA + 11.0mg D-mannitol. Centrifuge vial prior to opening. Reconstitute in 1ml sterile water for a concentration of 1µg/ml.
  ***Note:* Lyophilized components can be stored at -20ºC for up to a year from receipt. Reconstituted components are stable for 2 weeks when stored at 2-8°C. Aliquots of reconstituted components can be stored at -20°C for up to 6 months. Avoid more than one freeze-thaw cycle.**

HRP Conjugate:

- **Streptavidin-HRP Conjugate**:** 17µl vial. Expiration date on vial applies to unaliquoted material stored at 2-8ºC. Centrifuge vial prior to opening. Upon receipt, streptavidin-HRP conjugate should be diluted using 153µl of 1xPBS for a total of 170µl at a concentration of 100µg/ml. This solution can then be aliquoted into ten 16µl vials and stored at 2-8˚C for at least six months.
  Note: Store in Dark. Do Not Freeze. Streptavidin-HRP should be used in conjunction with TMB only.

RECOMMENDED MATERIALS

Available in PeproTech's
TMB ELISA Buffer Kit (Cat# 900-T00)

ELISA microplates: 96-well, flat-bottom
(Nunc MaxiSorp Prod. # 439454 or Corning Prod. # 3590)
Tween-20 (Sigma Cat. # P-7949)
BSA (Sigma Cat. # A-7030)
TMB Substrate (KPL Product Code #52-00-02)
Dulbecco's PBS [10x] (Gibco BRL Cat. # 14200-075)

RECOMMENDED SOLUTIONS

All solutions should be at ambient temperature prior to use.

PBS: dilute 10xPBS to 1xPBS, pH 7.20 in sterile water.
Wash Buffer: 0.05% Tween-20 in PBS
Block Buffer: 1% BSA in PBS *
Diluent: 0.05% Tween-20, 0.1% BSA in PBS *
Sterile filter and store at 4˚C for up to 1 week.

PLATE PREPARATION

1. Dilute capture antibody with PBS to a concentration of 0.50µg/ml. Immediately, add 100µl to each ELISA plate well. Seal the plate and incubate overnight at room temperature.
2. Aspirate the wells to remove liquid and wash the plate 4 times using 300µl of wash buffer per well. After the last wash invert plate to remove residual buffer and blot on paper towel.
3. Add 300µl block buffer to each well. Incubate for at least 1 hour at room temperature.
4. Aspirate and wash plate 4 times.

ELISA PROTOCOL

Standard/Sample:
Dilute standard from 2000pg/ml to zero in diluent. Immediately add 100µl of standard or sample to each well in triplicate. Incubate at room temperature for at least 2 hours.

Detection:
Aspirate and wash plate 4 times. Dilute detection antibody in diluent to a concentration of 0.125µg/ml. Add 100µl per well. Incubate at room temperature for 2 hours.

Streptavidin-HRP Conjugate:
Aspirate and wash plate 4 times. Dilute Streptavidin-HRP in diluent to a concentration of 0.10µg/ml. Add 100µl per well. Incubate 30 minutes at room temperature.

TMB Liquid Substrate:
(TMB Substrate should be at ambient temperature prior to use)
Aspirate and wash plate 4 times. Add 100µl of substrate solution to each well. Incubate at room temperature for color development for 20 minutes. Add 100µl of 1M HCl Stop Solution.
Monitor color development with an ELISA plate reader at 450nm with wavelength correction set at 620nm.

NOTE: Reliable standard curves are obtained when O.D. readings do not exceed 0.15 units for the zero standard concentrations. *O.D. readings may vary.*

CROSS REACTIVITY

When tested at 50ng/ml the following antigens did not exhibit significant cross reactivity:
Human: sIL-6 Receptor alpha, IL-11, Cardiotrophin-1, Oncostatin-M (196a.a.), Oncostatin-M (209a.a.) and Oncostatin-M (227a.a.)
Murine: IL-6 and IL-11, Cardiotrophin-1
Rat: IL-6