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CERTIFICATE OF ANALYSIS

Rat TNF-alpha ELISA Matched Antibody Pair Kit

Catalog Number: OOTA00710

Lot Number: KF0489

PRODUCT INFORMATION

DESCRIPTION

Rat TNF-α ELISA Matched Antibody Pair Kit contains the key components required for the quantitative measurement of natural and/or recombinant Rat TNF-α in a sandwich ELISA format within the range of 47-6000 pg/ml. Using the ELISA protocol described below, the recommended microplates, reagents and solutions, the components supplied in this kit are sufficient to assay Rat TNF-α in approximately 200 ELISA plate wells.

RECONSTITUTION & STORAGE

Capture Antibody*: 6 µg of Rabbit Anti-Rat TNF-α + 0.5 mg D-mannitol. Centrifuge vial prior to opening. Reconstitute in 60 µl sterile water for a concentration of 100 µg/ml.

Detection Antibody*: 11 µg of Biotinylated Rabbit Anti-Rat TNF-α + 0.5 mg D-mannitol. Centrifuge vial prior to opening. Reconstitute in 110 µl sterile water for a concentration of 100 µg/ml.

Rat TNF-α Standard*: 1 µg of Recombinant-Rat TNF-α + 2.2 mg BSA + 11.0 mg D-mannitol. Centrifuge vial prior to opening. Reconstitute in 1 ml sterile water for a concentration of 1 µg/ml.

Note: Lyophilized components can be stored at -20°C 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.

Streptavidin-HRP Conjugate: 4 µl vial. Expiration date on vial applies to unaliquoted material stored at 2-8°C. Centrifuge vial prior to opening. Upon receipt, streptavidin-HRP conjugate should be diluted using 36 µl of 1xPBS for a total of 40 µl at a concentration of 100 µg/ml. This solution can then be aliquoted into two 20 µl vials and stored at 2 -8°C for at least six months.**

Note: Store in Dark. Do Not Freeze. Streptavidin-HRP should be used

RECOMMENDED MATERIALS

ELISA Microplates (Nunc MaxiSorp Cat. No. 439454 or Corning Cat. No. 3590)
Tween-20 (Sigma Cat. No. P-7949)
BSA (Sigma Cat No. A-7030)
TMB Substrate (KPL Cat. No. 52-00-02)
Dulbecco's PBS [10x] (Gibco BRL Cat. No. 14200-075)
Sealing Film

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 0.25 µg/ml. Immediately, add 100 µl 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 300 µl of wash buffer per well. After the last wash invert plate to remove residual buffer and blot on paper towel.
3. Add 300 µl 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 6000 pg/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 0.25 µg/ml. Add 100 µl 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 0.075 ug/ml. Add 100 µl 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 100 µl of substrate solution to each well. Incubate at room temperature for color development for 20 minutes. Add 100 µl 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 exhibited less than 10% cross reactivity: Murine TNF-α When tested at 50ng/ml the following antigens exhibited less than 1% cross reactivity: Human TNF-α When tested at 50ng/ml the following antigens did not exhibit significant cross reactivity: Human MCP-1, TNF-β, sTNF Receptor Type I, sTNF Receptor Type II Rat CNTF, GM-CSF, IFN-γ, IL-13, IL-1α, IL-1β, MCP-1, SCF

Aviva Systems Biology guarantees the performance of this product for one year (12 months) from the date of receipt by the end user, or until the expiration date – whichever occurs first. This guarantee is dependent upon proper storage and handling as instructed on our Data Sheets and Certificates of Analysis. Every lot of product is quality tested.

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