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Human IL-1α
ELISA Development Kit
900-K11 Lot# 10011
Expiration one year from date of receipt

Description: Human IL-1α ELISA development kit contains the key components required for the quantitative measurement of natural and/or recombinant hIL-1α in a sandwich ELISA format within the range of 8–1000pg/ml. Using the ELISA protocol described below, the recommended microplates, reagents and solutions, the components supplied in this kit are sufficient to assay hIL-1α in approximately 600 ELISA plate wells.

RECONSTITUTION & STORAGE

Capture Antibody: 75µg of antigen-affinity purified rabbit anti-hIL-1α. Reconstitute in 0.75ml sterile water for a concentration of 100µg/ml.
Detection Antibody: 50µg of biotinylated antigen-affinity purified rabbit anti- hIL-1α. Reconstitute in 0.5ml sterile water for a concentration of 100µg/ml.
Human IL-1α Standard: 128ng of recombinant hIL-1α. Reconstitute in 1ml sterile water for a concentration of 128ng/ml.
Note: Reconstituted solutions of capture antibody and detection antibody can be kept at 4°-8°C for up to 2 months, or aliquoted and store frozen at ≤ -20°C for up to 6 months. Reconstituted solutions of the standard can be kept at 4°-8°C for up to 1 week, or aliquoted and store frozen at ≤ -20°C for up to 2 months.
Avidin Peroxidase Conjugate: 60µl vial. Upon receipt, avidin peroxidase conjugate should be aliquoted into ten 6µl vials and stored at ≤-20°C. Aliquots stored frozen at ≤-20°C are stable for up to 2 years from date of receipt.

RECOMMENDED MATERIALS (or purchase PeproTech's ELISA Buffer Kit: Cat. # 900-K00)

ELISA microplates (Nunc Maxisorp Prod. # 442404, or Corning Prod # 3590);
Tween-20 (Sigma Cat. # P-7949);
BSA (Sigma Cat # A-7030);
ABTS Liquid Substrate Solution (Sigma Cat. # A3219);
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 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 1µg/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 1ng/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 0.25µg/ml. Add 100µl per well. Incubate at room temperature for 2 hours.
Avidin Peroxidase: Aspirate and wash plate 4 times. Dilute one 6µl aliquot of Avidin Peroxidase 1:2000 in diluent for total volume of 12ml. Add 100µl 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 100µl of 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 either O.D. readings do not exceed 0.2 units for the zero standard concentrations, or 1.2 units for the highest standard concentration. The plate should be monitored at 5-minute intervals for approximately 25 minutes.

CROSS REACTIVITY

When tested at 50ng/ml the following antigens did not exhibit significant cross reactivity:

Human	IL-1β, IL-18
Murine	IL-1α, IL-1β, IL-18
Rat	IL-1β