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Human BD-4
ELISA Development Kit
900-K435 Lot# 0710435
Expiration one year from date of receipt

Description: Human BD-4 ELISA development kit contains the key components required for the quantitative measurement of natural and/or recombinant hBD-4 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 hBD-4 in approximately 1000 ELISA plate wells.

RECONSTITUTION & STORAGE

Capture Antibody: 25µg of antigen-affinity purified rabbit anti-hBD-4 + 2.5mg D-mannitol. Centrifuge vial prior to opening. Reconstitute in 0.25ml sterile water for a concentration of 100µg/ml.

Detection Antibody: 50µg of biotinylated antigen-affinity purified rabbit anti-hBD-4 + 2.5mg D-mannitol. Centrifuge vial prior to opening. Reconstitute in 0.5ml sterile water for a concentration of 100µg/ml.

Human BD-4 Standard: 1µg of recombinant hBD-4 + 2.2mg BSA + 11.0mg D-mannitol. Centrifuge vial prior to opening. Reconstitute in 1ml sterile water for a concentration of 1µg/ml.

Note: The reconstituted components are stable for 2 weeks when stored at 2-8°C. Components that have been reconstituted and aliquoted can be stored at -20°C for up to 6 months.

Avidin-HRP Conjugate: 60µl vial. Upon receipt, avidin-HRP 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. Avoid more than one freeze-thaw cycle. **Avidin should be used in conjunction with ABTS only**.

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

ELISA microplates (Nunc MaxiSorp Prod. # 439454, 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, 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 0.25µg/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 0.5µg/ml. Add 100µl per well. Incubate at room temperature for 2 hours.

Avidin-HRP Conjugate: Aspirate and wash plate 4 times. Dilute 5.5µl of avidin-HRP conjugate 1:2000 in diluent for a total volume of 11ml. 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. *O.D. readings may vary.*

CROSS REACTIVITY

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

Human BD-1 (36a.a.), BD-1 (47a.a.), BD-2, BD-3