



Human Beta-Defensin-2 (BD-2) ELISA Development Kit

Prod. No.: B428
Pkg. Size: 10 Plates

Description

Human BD-2 ELISA development kit contains the key components required for the quantitative measurement of natural and/or recombinant hBD-2 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 Human BD-2 in approximately 1000 ELISA plate wells.

Reconstitution

Capture Antibody: 25 µg of antigen-affinity purified goat Anti-human BD-2. Reconstitute in 0.25 ml sterile water for a concentration of 100 µg/ml.

Detection Antibody: 50 µg of biotinylated antigen-affinity purified goat Anti-Human BD-2. Reconstitute in 0.5 ml sterile water for a concentration of 100 µg/ml.

Human BD-2 Standard: 1 µg of recombinant Human BD-2. Reconstitute in 1 ml sterile water for a concentration of 1 µg/ml.

UltraAvidin Peroxidase Conjugate: 60 µl vial. Upon receipt, Avidin peroxidase conjugate should be aliquoted into ten 6 µl vials and stored at ≤-20°C. Aliquots stored frozen at ≤-20°C are stable for up to 2 years from date of receipt.

Storage and Stability

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.

Recommended Materials

- ELISA microplates (Nunc Maxisorp Prod. #442404);
- Tween-20 (Sigma Cat. # P-7949);
- BSA (Sigma Cat # A-7030);
- ABTS Liquid Substrate Solution (Leinco Cat. # A202);
- 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 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 1ng/ml to zero in diluent. Immediately add 100 μ l 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 0.5 μ g/ml. Add 100 μ l per well. Incubate at room temperature for 2 hours.

UltraAvidin Horseradish Peroxidase: Aspirate and wash plate 4 times. Dilute one 6 μ l aliquot of UltraAvidin Peroxidase 1:2000 in diluent for total volume of 12ml. Add 100 μ l per well. Incubate 30 minutes at room temperature.

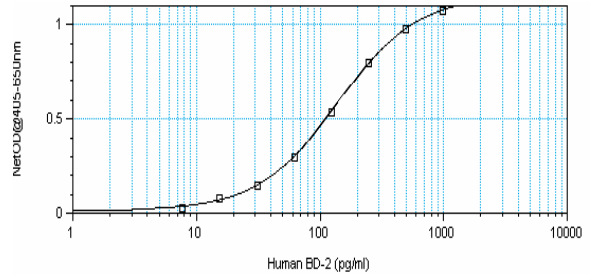
Substrate: Aspirate and wash plate 4 times. Add 100 μ l of substrate solution to each well. Incubate at room temperature for color development. Monitor color development with an ELISA plate reader at 405 nm with wavelength correction set at 650 nm.

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 15 minutes.

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-3



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