

REV: 1/13

ANTIGENIX AMERICA, INC.

PRODUCT INFORMATION SUMMARY

Human BD-2 (Beta Defensin) ELISA Construction Kit

Product Number RHF900CK
Approx. 960 tests

Product Number **RHF900CKC**
With Developing Reagents:

Capture Antibody 50.0 ug
Biotin-Labeled tracer 25.0 ug
Antigen Standard (see vial)

ELISA Coating Stabilizer 50 mL
Streptavidin-HRP 0.5 mL
TMB Substrate (50 mL x 2)
WASH Buffer (20X) 100 mL

DESCRIPTION:

This ELISA CONSTRUCTION Kit provides antigen affinity purified capture and tracer antibodies, and antigen standard sufficient for **approximately** ten microplates.

Working concentrations must be optimized by customer.

Note: Reconstitute components only when ready to run assay.

CAPTURE ANTIBODY: Provided as 50.0 ug lyophilized. Reconstitute with 500 uL distilled water.

TRACER ANTIBODY:

Provided as 25 ug (lyophilized)or as **0.5 mL liquid @ 50.0 ug/mL (see vial)** of Biotin labeled, antigen-affinity purified antibody, additive-free. Reconstitute the 25 ug (lyophilized vial) in 500 uL sterile water **containing 0.1% BSA.** (FREEZE aliquots for long-term storage). -

****For liquid vial store refrigerated only (contains preservative)**.**

STANDARD: Provided as 1.0 ug or 5.0 ug (**see vial**) of recombinant Human BD-2. Quick-spin and reconstitute in 100 uL of **sterile or distilled water.** Further dilutions can be made in 0.05% Tween-20, 0.1% BSA in PBS.

DEVELOPING REAGENTS: Supplied with catalog # ending in "CKC".

- ELISA Coating/ Blocking Reagent (EA150C) 50.0 mL (5X Solution)
- Streptavidin-HRP (S100180C) 0.5 mL - store @ -20 Deg. C.
- TMB Substrate Solutions - Part A and Part B (50.0 mL each) cat # ES200C
- Wash buffer (20X)- Dilute 1 part with 19 parts distilled water

HANDLING/ STORAGE: Reconstitute reagents when ready to build ELISA assay. Antibodies, after reconstitution - (Capture and Tracer) can be stored for approximately one month at 4 Degrees C. Or store **frozen** at -20 Degrees C. for up to 6 months.

For biotin tracer antibody provided as 0.5 mL - **STORE refrigerated** only - contains preservative.

Standard (rec. Human BD-2) can be stored in liquid state (@ 4 Deg. C.) For up to one week, or store **frozen, with addition of 0.1% BSA**, at -20 Deg. C. for up to 2 months. AVOID repeat freeze-thaw.

MATERIALS RECOMMENDED:

ELISA Microplates: Nunc Maxisorp, Prod. # 4420404
Tween -20.
BSA
Streptavidin-HRP: ANTIGENIX Cat no. **S100180** or similar
TMB Substrate (ANTIGENIX cat # **ES200**)
Dubelco's PBS (10X)
ANTIGENIX **ELISA Coating Stabilizer** (cat no: **EA150**)

RECOMMENDED SOLUTIONS:

Note: see ANTIGENIX Developing reagents above.
PBS: Dilute to 1XPBS in sterile water
WASH BUFFER: ANTIGENIX **WB200** or 0.05% Tween-20 in PBS.
BLOCK BUFFER: **use ANTIGENIX AMERICA coating stabilizer (EA150) or 1% BSA** in PBS
Substrate Solution: TMB Substrate Solution (ANTIGENIX # **ES200**)
Diluent: 0.1% BSA in PBS
2N Sulfuric acid (stop solution)

PLATE PREPARATION:

1. Dilute **portion** of capture antibody with 0.05M Carbonate buffer (or PBS) to concentration **0.50 ug/mL**.

Immediately add 100 uL to each ELISA well. Seal the plate and incubate overnight at room temperature.

2. Aspirate wells to remove all liquid and wash **4 times** using 300 uL of wash buffer per well. After last wash, add 200 uL ANTIGENIX AMERICA **ELISA coating stabilizer - recommended- (cat # EA150)** and incubate for 60 minutes at room temperature. (With coating stabilizer, **DO NOT** let plate dry prior to use of coating stabilizer. This will **stabilize and Block in one step!** Refer to data sheet EA150 for complete description of use.
3. With ANTIGENIX coating stabilizer (**recommended**) aspirate plate but **DO NOT WASH**. For extended storage- dry plate in humidity controlled chamber or similar. (see data sheet EA150). With standard block reagent, aspirate plate and wash

3X with 300 uL wash buffer.

PROTOCOL:

STANDARD/SAMPLE: Dilute a portion of the standard (store unused standard in aliquots, high concentration, frozen -20 Deg. C.) from **2.0 ng/mL** to zero in diluent (8-point, 1:2 -serial dilution). Immediately **add** 100 uL of standard or sample to each well in duplicate. **Incubate** at room temp. for approx. 2 hours.

DETECTION: Aspirate and wash plate 4 times. **Dilute** portion of the detection (Biotin Tracer) antibody in diluent to concentration of **0.25 ug/mL**. (Approx. 1:200 from 50.0 ug/mL). **Add** 100 uL per well. **Incubate** at room temperature for 1 hour. Note: detection antibody can be used in approximate range of 0.10 - 0.50 ug/mL, you may need to optimize for subsequent plates.

STREPTAVIDIN-HRP: Aspirate and wash plate 4 times. **Dilute** Streptavidin-HRP conjugate approx. **1:1,500** in diluent (follow recommended dilution of manufacturer). (May need to optimize) **Add** 100 uL per well, **incubate** 30 minutes at room temperature.

SUBSTRATE: Aspirate and wash plate 4 times. **Add** 100 uL substrate solution to each well. (follow directions from manufacturer) **Incubate** at room temp. for color development. Monitor color development with plate reader at 650 nm wavelength (blue color). **Stop** the color reaction after **10 - 20 minutes** by adding 100 uL of 2N Sulfuric acid to each well. Then, **read plate @ 450 nm** after addition of stop solution.

NOTE: reliable standard curves are obtained when O.D. readings do not exceed 0.25 units for the zero standard concentration, or 2.0 units for the highest standard concentration.

X-REACTIVITY DATA:

No measurable X-Reactivity was observed @ 40-50 ng/mL for following:

Human BD-1; BD3; BD-4

NOTE: Kit can be ordered with **developing reagents** (cat# ends in 'CKC') - see page 1.