

REV : 11/15

**ANTIGENIX AMERICA, INC.  
PRODUCT INFORMATION SUMMARY**

**Human BD-3  
(Beta Defensin)  
ELISA Construction Kit**

Product Number RHF771CK  
Approx. 960 tests

Product Number **RHF771CKC**  
**With Developing Reagents:**

<b>Capture Antibody</b>	<b>100.0 ug</b>	ELISA Coating Stabilizer	50 mL
Biotin-Labeled tracer	25.0 ug	Streptavidin-HRP	0.5mL
Antigen Standard	1.0 ug or 5.0ug	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 lyophilized, **100.0 ug**, additive-free. Reconstitute in 0.50 mL sterile water (200.0 ug/mL).

**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 of recombinant Human BD-3. Quick-spin and **reconstitute in 50 uL distilled or sterile water.** Further dilutions can be made in 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 (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. Standard ( rec. Human BD-3) 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:**

See **ANTIGENIX Developing Reagents above.**

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 Stabilizer/Block solution** ( cat no: **EA150**)

**RECOMMENDED SOLUTIONS:**

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 **1.0 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 **4.0 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** detection (Biotin Tracer) antibody in diluent to concentration of **0.20 ug/mL** (approx. 1:250 dilution from 50.0 ug/mL). **Add** 100 uL per well. **Incubate** at room temperature for approx. 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. **1:2,000** 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. ( for 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.

**NOTE:** Kit can be ordered with **developing reagents:** Catalog # ending in "CKC" - see page 1.

### **X-reactivity Data:**

**No** X-reactivity observed at 40-50 ng/mL with following factors:

Human BD-1, BD-2, BD-5

**RESEARCH USE ONLY -NOT For DIAGNOSTIC USE**