# ANTIGENIX AMERICA, INC.

## PRODUCT INFORMATION SUMMARY

Human BD-4

Product Number RHF633CKX

"SUPER X" ELISA Kit

5 Plate Kit ( 5 x 96 tests)

RHF633CKX2 2 plate kit ( 2 X 96 tests)
RHF633CKX1 single plate kit (96 tests)

## Items Provided:

**PRE-COATED ELISA Plates** (single, 2 or 5 plates)

Biotin-Labeled tracer 25.0 ug or 0.5 mL ( see vial)
Antigen Standard 5.0 ug or 1.0 ug ( see vial)

Streptavidin-HRP 0.5 mL

TMB Substrate 25 mL x 2 ( 15 mL - 2 plate) Wash Buffer Concentrate 125 mL (20X) ( 50 mL - 2 plate)

## **DESCRIPTION:**

Human Beta Defensin-4 (BD-4) **SUPER X ELISA Kit** provides two or five **PRE-COATED** microplates ( coated with antigen affinity purified capture antibody stabilized with our proprietary ELISA coating/blocking reagent. A biotin labeled tracer antibody, antigen standard, HRP developing reagents and wash buffer are included.

Reactivity with various sample types, including serum/plasma samples, is evaluated by customer.

Note: Reconstitute components only when ready to run assay.

#### 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 5.0 ug ( or 1.0 ug - see vial) of recombinant Human BD-4. Quick-spin andreconstitute with 100 uL sterile or distilled water to concentration approx. 100 ug/mL. Further dilutions can be made in 0.05% Tween-20, 0.1% BSA in PBS.

#### **DEVELOPING REAGENTS:**

- Streptavidin-HRP (S100180CX) 0.5 mL store @ -20 Deg. C.
- TMB Substrate Solutions Part A and Part B ( 25.0 mL each) cat # ES200CX
- Wash Buffer Concentrate (20X concentrate) Mix 1 volume of wash buffer with 19 volumes of distilled water. Stable for one month @ 4 Deg. C. once mixed to working volume.

  Concentrate may be stored at Room Temp.

**HANDLING/ STORAGE:** Reconstitute reagents when ready to build ELISA assay. Tracer Antibody provided as lyophilized, after reconstitution can be stored for approximately one month at 4 Degrees C. or store **frozen** at -20 Degrees C. for up to 6 months.

Biotin tracer antibody provided as 0.5 mL liquid -STORE refrigerated only- contains preservative.

**Standard** (rec. BD-4) 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. Precoated ELISA plates should be stored in sealed plastic bag with desiccant pack, and are stable for one year from date of receipt.

Store Streptavidin-HRP (S100180XH) frozen @ -20 Deg. C.

Store TMB solutions and wash buffer concentrate at 4 Deg. C. CAUTION: Substrate Solution B contains 20% acetone. FLAMMABLE. Keep away from sources of heat or flame.

## MATERIALS RECOMMENDED:

Tween -20.

BSA (ELISA grade only, protease-free)

Dubelco's PBS (10X)

PBS: Dilute to 1XPBS in sterile water

Diluent: use : 0.05% Tween-20, 0.1% BSA in PBS

2N Sulfuric Acid ( stop solution).

#### PLATE PREPARATION:

Plates are **pre-coated** with capture antibody and blocked/stabilized with ANTIGENIX proprietary ELISA coating stabilizer (EA150) and are **ready to use**!

Store plates refrigerated (@ 4 Deg. C.) in sealed plastic bags with desiccant pack. Plates can be stored for one year from data of receipt.

#### PROTOCOL:

STANDARD/SAMPLE: Dilute a portion of the standard (store unused standard in aliquots, high concentration, frozen -20 Deg. C. with addition of 0.1% BSA) from 1.0 ng/mL to zero in diluent (serial dilution). Immediately add 100 uL of standard or sample to each well in duplicate. Incubate at room temp. for 90 minutes.

**DETECTION:** Aspirate and wash plate 4 times. Dilute detection (Biotin Tracer) antibody in diluent to concentration of **0.10 ug/mL**. Add 100 uL per well. **Incubate** at room temperature for **30 minutes**.

STREPTAVIDIN-HRP: Aspirate and wash plate 4 times. Dilute Streptavidin-HRP conjugate approx. 1:2,000 in diluent. Add 100 uL per well, incubate 30 minutes at room temperature.

SUBSTRATE: **Prepare substrate solution** no more than 15 minutes before last incubation of assay: Mix one part TMB Solution A with one part TMB Solution B in a clean container. If, upon mixing, TMB solution turns blue - TMB solution is **contaminated-DO NOT USE.** Use mixed substrate solution **WITHIN 2 Hours, and AVOID DIRECT LIGHT.** 

Aspirate and wash plate 4 times. **Note:** Wash steps are critical! Add 100 uL substrate solution to each well. Incubate at room temp. for color development. **Add** 100 uL of **Stop solution** (2N Sulfuric Acid) after **approx.** 10 minutes to stop color development - gently tap plate to mix. **Read plate** at 450 nm within 30 minutes of 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 2.2 units for the highest standard concentration.

## X-REACTIVITY DATA:

**NO** measurable X-reactivty was observed @ 40-50 ng/mL with following factors:

Human BD-1, BD-2, BD-3

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE

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