REV: 7/15

ANTIGENIX AMERICA, INC.

PRODUCT INFORMATION SUMMARY

Human IL-1 Beta
ELISA Construction Kit

Product Number RHF8582CK
Approx. 960 tests

Capture Antibody	100.0 ug
Biotin-Labeled tracer	25.0 ug
Antigen Standard	2.0 ug

Product Number **RHF858CKC**
With Developing Reagents:

ELISA Coating Stabilizer	50 mL
Streptavidin-HRP	0.5mL
TMB Substrate	(50 mL x 2)
Wash Buffer (20X)	100.0 mL

DESCRIPTION:
This ELISA CONSTRUCTION Kit provides antigen affinity purified capture and tracer antibodies, and antigen standard sufficient for **approximately** ten microplates (10 x 96 tests). 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 of Biotin labeled, antigen-affinity purified antibody, additive-free. Reconstitute in 500 uL sterile water **containing 0.1% BSA.**

STANDARD: Provided as 2.0 ug of recombinant Human IL-1 Beta. Quick-spin and reconstitute in 100 uL of sterile water. Further dilutions can be made in diluent: 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)- Diluet 1 part with 19 parts distilled water

HANDLING/ STORAGE: Reconstitute reagents when ready to build ELISA assay. After reconstitution- Antibodies (Capture and Tracer) can be stored @ -20 Degrees C. for up to 6 months. Standard (rec. Human IL-1 Beta) 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:

PBS: Dilute to 1XPBS in sterile water
WASH BUFFER: ANTIGENIX WB200 or similar
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 (cat # EA150)-recommended!-** 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 5.0 ng/mL (**Adjust;** depending on desired range and sensitivity of first standard curve) to zero in diluent (1:2, 8 point- serial dilution). Immediately add 100 uL of standard or sample to each well in duplicate. **Incubate** at room temp. for approx 90 minutes.

DETECTION: Aspirate and wash plate 4 times. Dilute portion of detection (Tracer) antibody in diluent to concentration of 0.25 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 0.15 - 0.40 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. (blue color). The color reaction may be stopped after approx. 10- 20 minutes by adding 100 uL of 2N Sulfuric acid to each well. Read plate @ 450 nm within 30 minutes of addition of stop solution.

NOTE: reliable standard curves are obtained when O.D. readings do not exceed 0.30 units for the zero standard concentration, or 1.6 units for the highest standard concentration.

NOTE: Kit may be order with developing reagents (see page 1) - catalog # ends in **'CKC'**.

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE