5/20

PRODUCT INFORMATION SUMMARY

Human IL-1 Alpha
ELISA Construction Kit

Product Number RH100111CK		Product Number **RH100111CKC**	
Approx. 960 tests		**With Developing Reagents:**	
Capture Antibody	100.0 ug	ELISA Coating Stabilizer	50 mL
Biotin-Labeled tracer	25.0 ug	Streptavidin-HRP	0.5mL
Antigen Standard	2.0 ug	TMB Substrate	(50 mL x 2)
		WASH Buffer (20X)	100 mL

RH100111CKP w/ Developing reagents & 10 plates

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 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 Alpha. Quick-spin and reconstitute in 100 uL of 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 IL-1 Alpha) 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:

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 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 1.0 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. 90 minutes.

DETECTION: Aspirate and wash plate 4 times. Dilute portion of detection (Biotin Tracer) antibody in diluent to concentration of **0.20 ug/mL.** Add 100 uL per well. Incubate at room temperature for 30- 40 minutes. 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. **Stop** the color reaction after 10 - 15 minutes by adding 100 uL of 2N Sulfuric acid to each well. Then, **read plate @ 450 nm**.

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.

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE