ANTIGENIX AMERICA Inc.

PRODUCT INFORMATION SUMMARY

Human EPO
ELISA Construction Kit

Product Number RHF639CK		Product Number **RHF639CKC**	
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	10.0 ug	TMB Substrate	(50 mL x 2)

RHF639CKP - w/ Developing Reagents and 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 1.0 mL sterile water (100.0 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 10.0 ug of recombinant Human EPO. Quick-spin and reconstitute in 50 uL of distilled 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 EPO) can be stored in liquid state (@ 4 Deg. C.) For up to one week, or store **frozen** 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: 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 (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 8.0 ng/mL 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 (Biotin Tracer) antibody in diluent to concentration of 0.20 ug/mL. (1:250 dilution from 50.0 ug/mL)
Add 100 uL per well.

Incubate at room temperature for approx. 30 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** within 30 minutes after the 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 2.2 units for the highest standard concentration.

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE

Order with Developing Reagents: Cat# ends in 'CKC'

Order with Developing Reagents and ten (10) ELISA plates: Cat# ends in 'CKP'