

## PRODUCT INFORMATION SUMMARY

### Human EPO ELISA Construction Kit

Product Number RHF639CK  
Approx. 960 tests

Product Number **RHF639CKC**  
**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'**

ANTIGENIX AMERICA Inc.

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