

REV: 01/18

ANTIGENIX AMERICA Inc.

PRODUCT INFORMATION SUMMARY

Human SCF ELISA Construction Kit

Product Number RHF540CK
Approx. 960 tests

Product Number **RHF540CKC**
With Developing Reagents:

| | | | |
|-------------------------|-------------------|----------------------------|--------|
| Capture Antibody | 100.0 ug | ELISA Coating Stabilizer | 50 mL |
| Biotin tracer | 0.5 mL or 25.0 ug | Streptavidin-HRP | 0.5mL |
| Antigen Standard | 1.0 ug or 2.0 ug | TMB Substrate (50 mL x 2) | |
| | | WASH Buffer (20X) | 100 mL |

RHF540CKP w/ Developing Reagents & ten 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 (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 1.0 ug or 2.0 ug of recombinant Human SCF. (**see vial**). 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. Biotin Tracer antibody provided lyophilized 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. Human SCF) 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:

(**Note: 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 of plates: 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** detection (Biotin Tracer) antibody in diluent to concentration of 0.20 ug/mL. (approx 1:250 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. **Add** 100uL **stop solution** (2N Sulfuric acid) after approximately 10 minutes to stop color reaction. Then, **read plate @ 450 nm** within 30 minutes of addition of stoip solution.

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

X-REACTIVITY DATA:

X-Reactivity tested @ 40-50 ng/mL with following factors:

X-Reactivity observed with Rat SCF and Mouse SCF
(100% OD response) .

No measurable X-Reactivity observed with:

Human: M-SCF, G-CSF; GM-CSF; PDGF (AA, BB or AB); IL-1 alpha and beta ; IL-2, TPO, flt-3 Ligand

Mouse: M-CSF; G-CSF; GM-CSF.