ANTIGENIX AMERICA Inc.

PRODUCT INFORMATION SUMMARY

Human sTRAIL
ELISA Construction Kit

Product Number **RHF760CKC**
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	1.0 ug or 10.0ug	TMB Substrate (50 mL x 2)
		WASH Buffer (20X) 100 mL

RHF760CKP: w/ Developing Reagents & 10 ELISA 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 ug, additive-free. Reconstitute in 0.50 mL sterile or distilled water (200.0 ug/mL).

TRACER ANTIBODY:
Provided as 25 ug (lyophilized or as 0.5 mL liquid- **see vial**) of Biotin labeled, antigen-affinity purified antibody, additive-free. Reconstitute lyophilized vial in 500 uL sterile water **containing 0.1% BSA.**
For biotin tracer provided as 0.5 mL liquid- STORE refrigerated.

STANDARD: Provided as 10.0 ug of recombinant Human TRAIL. **Quick-spin** and reconstitute in 100 uL of sterile water. Further dilutions can be made in 0.1% BSA in PBS.

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 TRAIL) 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:

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: 0.05% Tween-20, 0.1% BSA in PBS

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 2.0 ng/mL (1:2, 8 point serial dilution). Immediately **add** 100 uL of standard or sample to each well in duplicate. **Incubate** at room temp. for aprrox. 90 minutes.

DETECTION: Aspirate and wash plate 4 times. **Dilute** detection (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 1 hour.
Note: detection antibody can be used in approximate range of 0.15 - 0.30 ug/mL, you may need to optimize for subsequent plates.

STREPTAVIDIN-HRP: Aspirate and wash plate 4 times. Dilute Streptavidin-HRP conjugate approx. 1:2000 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 color developmnet after approx. 10-15 minutes. Read plate at 450 nm within 30 minutes.

NOTE: reliable standard curves are obtained when O.D. readings do not exceed 0.30 units for the zero standard concentration, or 1.5 units for the highest standard concentration.

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE

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