

# ANTIGENIX AMERICA, INC.

## PRODUCT INFORMATION SUMMARY

### Human CTGF

Product Number **RHF461CKX**

### "SUPER X" ELISA Kit

5 Plate Kit ( 5 x 96 tests)

Product Number **RHF461CKX2** ( 2 x 96 tests)

Product Number **RHF461CKX1** ( 1 x 96 tests)

#### Items Provided:

**PRE-COATED ELISA Plates** (single, 2 or 5 plates)  
Biotin-Labeled tracer 25.0 ug or 0.5 mL ( see vial)  
Antigen Standard 5.0 ug or 1.0 ug ( see vial)  
Streptavidin-HRP 0.5 mL  
TMB Substrate 25 mL x 2 ( 15 mL - 2 plate)  
Wash Buffer Concentrate 125 mL ( 20X) ( 50 mL - 2 plate)

#### DESCRIPTION:

Human Connective Tissue Growth Factor (CTGF) **SUPER X ELISA Kit** provides **PRE-COATED** microplates coated with antigen affinity purified capture antibody stabilized with our proprietary ELISA coating/blocking reagent. A biotin labeled tracer antibody, antigen standard, HRP developing reagents and wash buffer are included.

Reactivity with various sample types, including serum/plasma samples, is evaluated by customer.

Note: Reconstitute components only when ready to run assay.

#### TRACER ANTIBODY:

Provided as 25 ug ( lyophilized ) or as **0.5 mL liquid @ 33.0 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 5.0 ug ( or 1.0 ug - see vial) of recombinant Human CTGF. Quick-spin and **reconstitute in distilled water** (pH 8.0) - concentration approx. 0.1 mg/mL. Further dilutions can be made in 0.05% Tween-20, 0.1% BSA in PBS.

**DEVELOPING REAGENTS:**

- Streptavidin-HRP ( S100180CX) 0.5 mL - **store @ -20 Deg. C.**
- TMB Substrate Solutions - Part A and Part B ( 25.0 mL each) cat # ES200CX
- Wash Buffer Concentrate (20X concentrate) Mix 1 volume of wash buffer with 19 volumes of distilled water. Stable for one month @ 4 Deg. C. once mixed to working volume. Concentrate may be stored at room temp.

**HANDLING/ STORAGE:** Reconstitute reagents when ready to build ELISA assay. Biotin tracer Antibody provided as lyophilized (25 ug) can be stored for approximately one month, after reconstitution 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. CTGF) 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. Pre-coated ELISA plates should be stored in sealed plastic bag with desiccant pack, and are stable for one year from date of receipt. If needed, additional components ( antigen standard or developing reagents) can be purchased as separate items to run additional stored plates - inquire.

**Store** Streptavidin-HRP ( S100180XH) **frozen** @ -20 Deg. C.

Store TMB solutions and wash buffer concentrate at 4 Deg. C.  
**CAUTION: Substrate Solution B contains 20% acetone. FLAMMABLE.**  
**Keep away from sources of heat or flame.**

**MATERIALS RECOMMENDED:**

Tween -20.  
BSA ( ELISA grade only, protease-free)  
Dubelco's PBS (10X)  
PBS: Dilute to 1XPBS in sterile water  
**Diluent:** use : 0.05% Tween-20, 0.1% BSA in PBS  
**2N Sulfuric acid** ( stop solution)

**PLATE PREPARATION:**

Plates are **pre-coated** with capture antibody and blocked/stabilized with ANTIGENIX proprietary ELISA coating stabilizer ( EA150) and are **ready to use!**

Store plates refrigerated ( @ 4 Deg. C.) in sealed plastic bags with desiccant pack. Plates can be stored for one year from data of receipt.

**PROTOCOL:**

**MODIFIED PROCEDURE: ( Add Standard/Sample and Biotin together)**

**PREPARE STANDARD/SAMPLE:** Dilute **a portion of the** standard ( store unused standard in aliquots, high concentration, frozen -20 Deg. C. with addition of 0.1% BSA) from **4.0 ng/mL** to zero in diluent (serial dilution).

**PREPARE Biotin DETECTION:** Dilute portion of the detection (Biotin Tracer) antibody in diluent to concentration of **0.30 ug/mL**.

**Add** 100 ul of each Standard ( or sample) **and** 50.0 uL of the Biotin Detection antibody to each well in duplicate.

**Incubate** at Room Temp. for **90 minutes**. Aspirate and WASH plate 4 Times.

**STREPTAVIDIN-HRP:** Dilute Streptavidin-HRP conjugate approx. **1:2,000** in diluent. **Add 100 uL** per well, **incubate** for **1 hour** at room temperature.

**SUBSTRATE: Prepare substrate solution** no more than 15 minutes before last incubation of assay: Mix one part TMB Solution A with one part TMB Solution B in a clean container. If, upon mixing, TMB solution turns blue - TMB solution is **contaminated- DO NOT USE**. Use mixed substrate solution **WITHIN 2 Hours, and AVOID DIRECT LIGHT**.

Aspirate and wash plate 4 times. **Note:** Wash steps are critical! **Add 100 uL substrate** solution to each well. **Incubate** at room temp. for color development. **Add 100 uL of Stop solution** ( 2N Sulfuric Acid) **after 15-20 minutes** to stop color development - gently tap plate to mix. **Read plate** at **450 nm** within 30 minutes of 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 1.6 units for the highest standard concentration.

**X-REACTIVITY DATA:**

The following factors were tested @ 40-50 ng/mL:

**No** measurable X-Reactivity was observed with following:

Human: IGF-I; IGF-II, IGF-BP1; CTGFL; BMP-4; TGF beta;  
WISP-1 and 3.