REV: 9/13

ANTIGENIX AMERICA, INC.

PRODUCT INFORMATION SUMMARY

Rat GRO/KC
ELISA Construction Kit

Product Number RRF427CK
Approx. 960 tests

Capture Antibody **100.0 ug**
Biotin tracer 25.0 ug or 50.0 ug
Antigen Standard 1.0 ug or 5.0 ug

Product Number **RRF427CKC**
With Developing Reagents:

ELISA Coating Stabilizer 50 mL
Streptavidin-HRP 0.5mL
TMB Substrate (50 mL x 2)
WASH Buffer (20X) 100 mL

DESCRIPTION:
Rat GRO/KC (CXCL1) ELISA CONSTRUCTION Kit provides antigen affinity purified capture and tracer antibodies, and antigen standard for development of **approximately** ten microplate assays. 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). (FREEZE aliquots for long-term storage)

TRACER ANTIBODY:
Provided as 25 ug **(** lyophilized **)or as 0.5 mL liquid @ 100.0 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 5.0 ug (**see vial**) of recombinant Rat GRO/KC. **Quick-spin** and reconstitute in 50 uL of sterile water (pH 7.2). Further dilutions can be made in 0.05% Tween-20, 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 provided lyophilized (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.

For biotin tracer provided as 0.5 mL liquid- STORE refrigerated only -contains preservative.

Standard (rec. Rat GRO/KC) 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.

MATERIALS RECOMMENDED:

ELISA Microplates: Nunc Maxisorp, Prod. # 4420404
Tween -20.
BSA
Streptavidin-HRP: ANTIGENIX Cat no. **S100180** or similar
TMB Substrate : ANTIGENIX cat # **ES200** or similar
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: ANTIGENIX WB200 or 0.05% Tween-20 in PBS.
BLOCK BUFFER: **use ANTIGENIX AMERICA coating stabilizer-recommended (EA150) or** 1% BSA in PBS
Substrate Solution: TMB Substrate Solution (cat # **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** to zero in diluent (serial dilution). Immediately add 100 uL of standard or sample to each well in duplicate. Incubate at room temp. for approx. 2 hours.

DETECTION: Aspirate and wash plate 4 times. Dilute detection (Tracer) antibody in diluent to concentration of 0.25 ug/mL. Add 100 uL per well. Incubate at room temperature for 1-2 hours. 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 PBS with 0.1% BSA as 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. Prepare and add 100 uL substrate solution (cat no: ES200)to each well (follow directions for substrate preparation and handling from manufacturer's data sheet). **Incubate** at room temp. for color development. Monitor color development with plate reader at 650 nm wavelength. (for blue color). Add 100 uL stop solution (2N Sulfuric acid) after 10-20 monutes to stop color development. Then, read plate @ 450 nm within 30 minutes of addition of stop solution.

NOTE: reliable standard curves are obtained when O.D. readings do not exceed 0.25 units for the zero standard concentration, or 1.6 units for the highest standard concentration.

NOTE: Kit can be ordered with developing reagents - cat# ends in 'CKC' - see page 1.

X-REACTIVITY DATA:

X-Reactivity (**100%**) observed @ 40-50 ng/mL with following factor: Mouse KC

Minimal (1% or less) X-reactivity observed with following:

Mouse MIP-2;
Rat GRO-beta

Human: GRO; GRO beta; GRO gamma