

ANTIGENIX AMERICA, INC.

PRODUCT INFORMATION SUMMARY

Human GRO-gamma ELISA Construction Kit

Product Number RHF820CK
Approx. 960 tests

Product Number **RHF820CKC**
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	2.0 ug	TMB Substrate (50 mL x 2)	
		WASH Buffer (20X)	100 mL

DESCRIPTION:

Human GRO-gamma 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 of Biotin labeled, antigen-affinity purified antibody, additive-free. Reconstitute in 500 uL sterile water **containing 0.1% BSA.** (FREEZE aliquots for long-term storage)

STANDARD: Provided as 2.0 ug of recombinant Human GRO-gamma. **Quick-spin** and reconstitute in 50 uL of sterile water (pH 7.2). 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 GRO-gamma) can be stored in liquid state (@ 4 Deg. C.) for up to one week, or store **frozen** at -20 Deg. C. (**Add 0.1% BSA**) for up to 3 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 (EA150)**
or 1% BSA in PBS
Substrate Solution: TMB Substrate Solution (cat # **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 10.0 ng/mL (**Adjust;** depending on desired range and sensitivity of first standard curve) 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** portion of 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. 1 hour. 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. **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). **Stop** the color reaction after **approx. 10 - 20 minutes** by adding 100 uL of 2N Sulfuric acid to each well. Then, **read plate @ 450 nm** after 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.

NOTE: Kit can be ordered with **developing reagents** -cat no. ends in 'CKC' - see page 1.

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE