

# ANTIGENIX AMERICA, INC.

## PRODUCT INFORMATION SUMMARY

### Human GRO-gamma ELISA Construction Kit

Product Number RHF820CK  
Approx. 960 tests

Product Number **RHF820CKC**  
**With Developing Reagents:**

<b>Capture Antibody</b>	<b>100.0 ug</b>	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**

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