

REV: 9/16

ANTIGENIX AMERICA Inc.

## PRODUCT INFORMATION SUMMARY

### Human I-309 ELISA CONSTRUCTION KIT

Product Number RHF830CK  
Approx. 960 tests

Product Number **RHF830CKC**  
**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

#### **RHF830CKP w/ Developing reagents & 10 plates**

#### **DESCRIPTION:**

Human I-309, also known as **CCL1**. This ELISA Construction kit supplies the main components to develop ELISA assay for human I-309.

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).

#### **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**.

**STANDARD:** Provided as 2.0 ug of recombinant Human I-309. Quick-spin and reconstitute in 50 uL of sterile or distilled water. 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. Reconstituted 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. I-309) 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:**

See **ANTIGENIX Developing Reagents** above.

ELISA Microplates: Nunc Maxisorp, Prod. # 4420404  
Tween -20.

BSA

Streptavidin-HRP: ANTIGENIX Cat no. **S100180** or similar

TMB Substrate, ANTIGENIX cat. No. **ES200**

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

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

**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 dilution from 50.0 ug/mL)

**Add** 100 uL per well. **Incubate** at room temperature for 40 minutes - 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. **Add** 100 uL substrate solution to each well. **Incubate** at room temp. for color development. **Stop** the color reaction after 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 2.0 units for the highest standard concentration.

**NOTE:** Kit can be ordered with developing reagents ( cat# ends in 'CKC') - or with Developing reagents and 10 blank ELISA plates RHF830CKP- see page 1.

**RESEARCH USE ONLY -NOT For DIAGNOSTIC USE**

ANTIGENIX AMERICA Inc.

[WWW.ANTIGENIX.COM](http://WWW.ANTIGENIX.COM)