

REV: 4/16

ANTIGENIX AMERICA INC.

PRODUCT INFORMATION SUMMARY

Human IFN Beta ELISA Construction Kit

Product Number RHF842CK
Approx. 960 tests

Product Number **RHF842CKC**
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 | 5.0 ug | TMB Substrate (50 mL x 2) | |
| | | WASH Buffer (20X) | 100 mL |

DESCRIPTION:

This ELISA CONSTRUCTION Kit provides antigen affinity purified capture and tracer antibodies, and antigen standard sufficient for **approximately** ten microplates.
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).

TRACER ANTIBODY:

Provided as 25 ug (lyophilized or as 0.5 mL liquid- **see vial**) of Biotin labeled, antigen-affinity purified antibody, additive-free. Reconstitute lyophilized vial in 500 uL sterile water **containing 0.1% BSA**.
For biotin tracer provided as 0.5 mL liquid- STORE refrigerated.

STANDARD: Provided as 5.0 ug of recombinant Human IFN Beta. Quick-spin and reconstitute in 100 uL of sterile water. Further dilutions can be made in diluent - 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.
Biotin tracer provided as 0.5 mL - STORE refrigerated only.

Standard (rec. Human IFN Beta) 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)
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 (ANTIGENIX # ES200)
Diluent: use 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 **4.0 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. **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**. 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 PBS/0.1% BSA 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. (follow directions from manufacturer) 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** after **10-15 minutes** to stop color development. **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 0.30 units for the zero standard concentration, or 1.6 units for the highest standard concentration.

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

REFERENCES:

1) Anna-Maria Herzner, Martin Schlee, et al; G-rich DNA-induced stress response blocks type-I IFN but not CKCL10 secretion in monocytes; Nature Scientific Reports (6); 12 Dec. 2016; article 38405, doi:10.1038/srep38405

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