ANTIGENIX AMERICA Inc.

# PRODUCT INFORMATION SUMMARY

## Human NNT-1 / BCSF-3

Product Number **RHF778CKX**

## "SUPER X" ELISA Kit

5 Plate Kit ( 5 x 96 tests)
**RHF778CKX2** 2 plate kit ( 2 X 96 tests)
**RHF778CKX1** ( single plate)

**Items Provided:**

**PRE-COATED ELISA Plates** (single, 2 or 5 plates)

| | |
|---|---|
| Biotin-Labeled tracer | 25.0 ug **or 0.5 mL ( see vial)** |
| Antigen Standard | 2.0 ug |
| Streptavidin-HRP | 0.5 mL |
| TMB Substrate | 25 mL x 2 ( 15 mL - 2 plate) |
| Wash Buffer Concentrate | 125 mL (20X) ( 50 mL - 2 plate) |

Diluent (10X) and Stop Solution (1X)

**DESCRIPTION:**

Human NNT-1 / BCSF-3 (Novel Neurtrophin-1/B-Cell-Stimulating Factor-3) **SUPER X ELISA Kit** provides **PRE-COATED** microplates coated with antigen affinity purified capture antibody stabilized with our proprietary ELISA coating/blocking reagent. A biotin labeled tracer antibody, antigen standard, HRP developing reagents and wash buffer are included.

Note: Reconstitute components only when ready to run assay.

**TRACER ANTIBODY:**

Provided as 25 ug **(** lyophilized **)or as 0.5 mL liquid @ 50.0 ug/mL** ( **see vial)** of Biotin labeled, antigen-affinity purified antibody, additive-free. Reconstitute the 25.0 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 2.0 ug of recombinant Human NNT-1.

Quick-spin and **reconstitute with 50.0 uL distilled water .** Further dilutions can be made in ELISA Diluent provided or 0.1% BSA in PBS.

**DEVELOPING REAGENTS:**

- Streptavidin-HRP ( S100180CX) 0.5 mL - **store** @ **-20 Deg. C.**
- TMB Substrate Solutions - Part A and Part B ( 25.0 mL each) cat # ES200CX
- Wash Buffer Concentrate (20X concentrate) Mix 1 volume of wash buffer with 19 volumes of distilled water. Stable for one month @ 4 Deg. C. once mixed to working volume.
  Concentrate may be stored at Room Temp.
- Diluent (10X) - Mix 1 part with 9 parts distilled water.
- STOP Solution (1X) -use neat ( CAUTION: **CAUSTIC**)

**HANDLING/ STORAGE:** Reconstitute reagents when ready to build ELISA assay. Tracer Antibody provided as 25.0 ug -lyophilized, after reconstitution- be stored for approximately one month at 4 Degrees C. Or store **frozen** at -20 Degrees C. for up to 6 months.

Biotin tracer antibody provided as 0.5 mL liquid- STORE refrigerated only - contains preservative.

Standard ( rec. NNT-1) 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. Pre-coated ELISA plates should be stored in sealed plastic bag with desiccant pack, and are stable for one year from date of receipt.

**Store** Streptavidin-HRP ( S100180X) **frozen** @ -20 Deg. C.

Store TMB solutions and wash buffer concentrate at 4 Deg. C.
**CAUTION: Substrate Solution B contains 20% acetone. FLAMMABLE. Keep away from sources of heat or flame.**

**MATERIALS RECOMMENDED:**

Tween -20.
BSA ( ELISA grade only, protease-free)
Dubelco's PBS (10X)
PBS: Dilute to 1XPBS in sterile water

**PLATE PREPARATION:**

Plates are **pre-coated** with capture antibody and blocked/stabilized with ANTIGENIX proprietary ELISA coating stabilizer ( EA150) and are **ready to use**!

Store plates refrigerated ( @ 4 Deg. C.) in sealed plastic bags with desiccant pack. Plates can be stored until expiration date on kit box.

**PROTOCOL:**

**STANDARD/SAMPLE: Dilute a portion of the** standard ( store unused standard in aliquots, high concentration, frozen -20 Deg. C. with addition of 0.1% BSA) 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 **90** minutes

**DETECTION:** Aspirate and wash plate 4 times. **Dilute** detection (Biotin Tracer) antibody in diluent to **concentration** of **0.2 ug/mL.** ( approx. 1:250 from 50.0 ug/mL) Add 100 uL per well. **Incubate** at room temperature for **30 – 40 minutes.**

**STREPTAVIDIN-HRP:** Aspirate and wash plate 4 times. **Dilute** Streptavidin-HRP conjugate approx. **1:2,500** in diluent. (May need to optimize) Add 100 uL per well, **incubate 30 minutes** at room temperature.

**SUBSTRATE: Prepare substrate solution** no more than 15 minutes before last incubation of assay: Mix one part TMB Solution A with one part TMB Solution B in a clean container. If, upon mixing, TMB solution turns blue - TMB solution is **contaminated- DO NOT USE.** Use mixed substrate solution **WITHIN 2 Hours, and AVOID DIRECT LIGHT.**

Aspirate and wash plate 4 times. **Note:** Wash steps are critical! **Add** 100 uL substrate solution to each well. **Incubate** at room temp. for color development. **Add** 100 uL of **Stop solution** ( 2N Sulfuric Acid) **after approx. 10 minutes** to stop color development - gently tap plate to mix. **Read plate** at **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 2.0 units for the highest standard concentration.

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