

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

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