

# ANTIGENIX AMERICA, INC.

## PRODUCT INFORMATION SUMMARY

**Human LD78-Beta**  
**"SUPER X" ELISA Kit**  
(CCL3L1)

Product Number **RHF927CKX**  
5 Plate Kit ( 5 x 96 tests)  
**RHF927CKX2** ( 2 x 96 tests)  
**RHF927CKX1** ( 96 tests)

### 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 5.0 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)

### DESCRIPTION:

Human LD78-Beta ( **also known as CCL3L1**) **SUPER X ELISA Kit** provides single, two or five **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.

Reactivity with various sample types, including serum/plasma samples, is evaluated by customer.

Note: Reconstitute components only when ready to run assay.

### TRACER ANTIBODY:

Provided as 25 ug lyophilized or 0.5 mL liquid ( see vial) of Biotin labeled, antigen-affinity purified antibody, additive-free. For 25.0 ug lyophilized : Reconstitute in 200 uL sterile water **containing 0.1% BSA.** ( FREEZE aliquots for long-term storage)

For liquid vial ( 0.5 mL) - store refrigerated only.

**STANDARD:** Provided as 5.0 ug of recombinant Human LD78-Beta. Quick-spin and **reconstitute in 50 uL distilled water** (pH 8.0) - Further dilutions can be made in Diluent provided (ED100) 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) - 30 mL or 15 mL - Dilute 1 part with 29 parts distilled water or PBS solution.
- STOP Solution (1X) - CAUTION - CAUSTIC ( if provided)

**HANDLING/ STORAGE:** Reconstitute reagents when ready to build ELISA assay. Reconstituted Tracer Antibody be stored for approximately one month at 4 Degrees C. Or store **frozen** at -20 Degrees C. for up to 6 months.

For tracer antibody ( 0.5 mL) provided liquid- store refrigerated only- contains preservative.

Standard ( rec. LD78-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. Pre-coated ELISA plates should be stored refrigerated ( @ 4 Deg.C.) in sealed plastic bag with desiccant pack, and are stable until expiration date on kit box.

**Store** Streptavidin-HRP ( S100180XH) **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  
**Diluent:** use : ANTIGENIX ED100 or 0.1% BSA in PBS

**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.

**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 **1.0 ng/mL ( 1,000.0 pg/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 **1 hour**.

**DETECTION:** Aspirate and wash plate 4 times. **Dilute** 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 **1 hour**.

**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** ( ANTIGENIX ED100) **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 0.20 units for the zero standard concentration, or 2.5 units for the highest standard concentration.

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

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