REV 1/18

ANTIGENIX AMERICA Inc.

PRODUCT INFORMATION SUMMARY

Human Galectin-3 "SUPER X" ELISA Kit

Product Number **RHF332CKX**
5 Plate Kit (5 x 96 tests)
2 Plate kit Product Number **RHF332CKX2**
RHF332CKX1 (96 tests)

Items Provided:

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

Biotin-Labeled tracer	25.0 ug
Antigen Standard	10.0 ug
Streptavidin-HRP	0.5 mL -
TMB Substrate	25 mL x 2 (15 mL - 2 plate)
Wash Buffer Concentrate	125 mL (20X);or 50 mL (2 plate kit)

DESCRIPTION:

Human Galectin-3 **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 , and 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 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 10.0 ug of recombinant Human Galectin-3. Quick-spin and **reconstitute in distilled water** (pH 8.0) - concentration approx. 0.1 mg/mL. Further dilutions can be made in diluent provided or 0.1% BSA in PBS.

DEVELOPING REAGENTS:

- Streptavidin-HRP (S100180CX) 0.5 mL 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.
- Diluent (10X) -Dilute 1 part with 9 parts distilled water
- STOP Solution (1X) 2N Sulfuric Acid - **CAUTION - CAUSTIC**

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

For biotin tracer provided as 0.5 mL liquid- STORE refrigerated only- contains preservative.

Standard (rec. Galectin-3) 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 until expiration date on kit box.

Store Streptavidin-HRP (S100180CX) @ -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 : provided 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. 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 2.0 ng/mL to zero blank 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 (Tracer) antibody in diluent to concentration of 0.20 ug/mL. Add 100 uL per well. **Incubate** at room temperature for 40 minutes .

STREPTAVIDIN-HRP: Aspirate and wash plate 4 times. **Dilute** Streptavidin-HRP conjugate approx. 1:2,000 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) within 10-15 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.