ANTIGENIX AMERICA, INC.

REV. 5/13

PRODUCT INFORMATION SUMMARY

Human PF-4
"SUPER X" ELISA Kit

Product Number **RHF580CKX**
5 Plate Kit (5 x 96 tests)

RHF580CKX2 (2 Plate Kit)

Items Provided:

PRE-COATED ELISA Plates (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 Platelet factor-4 (PF-4) **SUPER X ELISA Kit** provides 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)@ 50.0 ug/mL - see vial -** of Biotin labeled, antigen-affinity purified antibody.

Reconstitute 25.0 ug lyophilized vial in 500 uL sterile water **containing 0.1% BSA**. (FREEZE aliquots for long-term storage).

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

STANDARD: Provided as 5.0 ug of recombinant Human PF-4. Quick-spin and **reconstitute in distilled water** (pH 8.0) - concentration approx. 0.1 mg/mL. Further dilutions can be made in 0.05% Tween-20, 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.

HANDLING/ STORAGE:

Reconstitute reagents when ready to build ELISA assay. Tracer Antibody (25.0 ug) reconstituted from lyophilized - can be stored for approximately one month at 4 Degrees C. Or store **frozen** at -20 Degrees C. for up to 6 months. Tracer antibody (0.5 mL) provided liquid- STORE refrigerated only.

Standard (rec. PF-4) 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.

Store Streptavidin-HRP (S100180CX) **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 : 0.05% Tween-20, 0.1% BSA in PBS
2N Sulfuric acid - Stop Solution.

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 for one year from data of receipt.

PROTOCOL:

MODIFIED PROCEDURE: (Add Standard/Sample and Biotin together)

PREPARE 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 **10.0 ng/mL** to zero in diluent (serial dilution).

PREPARE Biotin DETECTION: Dilute portion of the detection (Biotin Tracer) antibody in diluent to concentration of **0.10 ug/mL**.

Add 100 ul of each Standard (or sample) **and** 50.0 uL of the Biotin Detection antibody to each well in duplicate.

Incubate at Room Temp. for **60 minutes.** Aspirate and WASH plate 4 Times.

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 0.25 units for the zero standard concentration, or 2.0 units for the highest standard concentration. Monitor the plate every 5 minutes for approximately 30 minutes.