

ANTIGENIX AMERICA Inc.

## PRODUCT INFORMATION SUMMARY

### Human p16-INK4a-TAT

### "SUPER X" ELISA Kit

Product Number **RHF857CKX**

5 Plate Kit ( 5 x 96 tests)

**RHF857CKX2** ( 2 plate kit)

**RHF857CKX1** ( 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 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 p16-INK4a-TAT **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 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 5.0 ug of recombinant Human p16-INK4a. Quick-spin and **reconstitute in distilled water** (pH 8.0) - concentration approx. 0.1 mg/mL. Further dilutions can be made in diluent ( ANTIGENIX 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.
- Diluent (10X) - Dilute 1 part with 9 parts distilled water

**HANDLING/ STORAGE:** Reconstitute reagents when ready to build ELISA assay. Biotin Tracer Antibody provided as 25.0 ug lyophilized - can 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. RELM 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 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  
2N Sulfuric acid ( **stop solution**) or ANTIGENIX STP100

#### 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 10.0 ng/mL** to zero in diluent (8-point, 1:2 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 **0.20 ug/mL**. ( Approx. 1:250 from 50.0 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) after **approx. 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:** TMB incubation may be as little as **10 minutes**.

NOTE: reliable standard curves are obtained when O.D. readings do not exceed 0.30 units for the zero standard concentration, or 1.6 units for the highest standard concentration.

**NOTE:** \*\* Always refer to protocol/ data sheet provided with kit ordered/ delivered , as **working dilutions may be lot sensitive** \*\*

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