

REV: 12/18

ANTIGENIX AMERICA Inc.

## PRODUCT INFORMATION SUMMARY

### Human p16-INK4a-TAT ELISA Construction Kit

Product Number RHF857CK  
Approx. 960 tests

Product Number **RHF857CKC**  
**With Developing Reagents:**

Capture Antibody	100.0 ug	ELISA Coating Stabilizer	50 mL
Biotin-Labeled tracer	25.0 ug	Streptavidin-HRP	0.5mL
Antigen Standard	5.0 ug	TMB Substrate ( 50 mL x 2)	
		Wash Buffer (20X)	100 mL

**RHF857CKP** w/ developing reagents and ten plates

#### DESCRIPTION:

This ELISA CONSTRUCTION Kit provides antigen affinity purified capture and tracer antibodies, and antigen standard sufficient for **approximately** ten microplates. ( 10 x 96 tests)  
Working concentrations must be optimized by customer.

Note: Reconstitute components only when ready to run assay.

#### CAPTURE ANTIBODY:

Provided as lyophilized, 100.0 ug, additive-free. Reconstitute in 1.0 mL sterile or distilled water (0.1 mg/mL).

#### 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-TAT. **Quick-spin** and reconstitute in 50 uL of sterile water. Further dilutions can be made in 0.1% BSA in PBS.

**DEVELOPING REAGENTS:** Supplied with catalog # ending in "CKC".

- ELISA Coating/ Blocking Reagent ( EA150C) 50.0 mL ( 5X Solution)
- Streptavidin-HRP ( S100180C) 0.5 mL - **store @ -20 Deg. C.**
- TMB Substrate Solutions - Part A and Part B ( 50.0 mL each)
- Wash Buffer (20X) -Dilute 1 part with 19 parts distilled water

**HANDLING/ STORAGE:** Reconstitute reagents when ready to build ELISA assay. Antibodies (Capture and Tracer) can be stored for approximately one week at 4 Degrees C. Or store **frozen** at -20 Degrees C. for up to 6 months.  
Biotin tracer provided as 0.5 mL liquid - STORE refrigerated.

Standard ( rec. Human p16-INK4a-TAT) can be stored in liquid state ( @ 4 Deg. C.) For up to one week, or store **frozen** at -20 Deg. C. ( **Add 0.1% BSA**) for up to 2 months. AVOID repeat freeze-thaw.

**MATERIALS RECOMMENDED:**

ELISA Microplates: Nunc Maxisorp, Prod. # 4420404  
Tween -20.  
BSA  
Streptavidin-HRP: ANTIGENIX Cat no. **S100180** or similar  
TMB Substrate (ANTIGENIX cat # **ES200**)  
Dubelco's PBS (10X)  
ANTIGENIX **ELISA Coating Stabilizer** ( cat no: **EA150**)  
**Wash Buffer:** ANTIGENIX cat# WB200

**RECOMMENDED SOLUTIONS:**

**See ANITGENIX Developing Reagents above.**

PBS: Dilute to 1XPBS in sterile water  
WASH BUFFER: 0.05% Tween-20 in PBS. Or ANTIGENIX WB200  
BLOCK BUFFER: **use ANTIGENIX AMERICA coating stabilizer - recommended! -(EA150) or 1% BSA in PBS**  
Substrate Solution: TMB Substrate Solution (ANTIGENIX # ES200)  
Diluent: 0.1% BSA in PBS  
2N Sulfuric acid ( stop solution).

**PLATE PREPARATION:**

1. Dilute **portion** of capture antibody with 0.05M Carbonate buffer (or PBS) to concentration 1.0 ug/mL.

Immediately add 100 uL to each ELISA well. Seal the plate and incubate overnight at room temperature.

2. Aspirate wells to remove all liquid and wash **4 times** using 300 uL of wash buffer per well. After last wash, add 200 uL ANTIGENIX AMERICA **ELISA coating stabilizer - recommended! -(cat # EA150)** and incubate for 60 minutes at room temperature. ( With coating stabilizer, DO NOT let plate dry prior to use of coating stabilizer. This will **stabilize and Block in one step!** Refer to data sheet EA150 for complete description of use.
3. With ANTIGENIX coating stabilizer ( **recommended** ) aspirate plate but **DO NOT WASH**. For extended storage, -dry plate in humidity controlled chamber or similar. ( see data sheet EA150). With standard block reagent, aspirate plate and wash 3X with 300 uL wash buffer.

**PROTOCOL:**

**STANDARD/SAMPLE:** Dilute a portion of the standard ( store unused standard in aliquots, high concentration, frozen -20 Deg. C.) from 8.0 ng/mL to zero blank: ( 8 point- 1:2 serial dilution) in diluent Immediately **add** 100 uL of **standard** or sample to each well in duplicate. **Incubate** at room temp. for approx. 90 minutes.

**DETECTION:** Aspirate and wash plate 4 times. **Dilute** a portion of detection (Tracer) antibody in diluent to concentration of 0.20 ug/mL ( 1:250 dilution from 50.0 ug/mL). **Add** 100 uL per well. **Incubate** at room temperature for approx. 40 minutes -1 hour. Note: detection antibody can be used in approximate range of 0.25 - 0.50 ug/mL, you may need to optimize for subsequent plates.

**STREPTAVIDIN-HRP:** Aspirate and wash plate 4 times. **Dilute** Streptavidin-HRP conjugate approx. 1:2,000 in diluent ( follow recommended dilution of manufacturer). (May need to optimize) **Add** 100 uL per well, incubate 30 minutes at room temperature.

**SUBSTRATE:** Aspirate and wash plate 4 times. Add 100 uL substrate solution to each well. ( follow directions from manufacturer) **Incubate** at room temp. for color development. Monitor color development with plate reader at 650 nm wavelength. ( for blue color). **STOP** the color reaction after 10 - 15 minutes by adding 100 uL of 2N Sulfuric acid to each well. Then, **read plate @ 450 nm** after 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**

**NOTE:** Kit can be ordered with developing reagents:  
cat # ends in 'CKC' -see page 1.

Or with Developing reagents and ten blank plates 'CKP'