REV: 4/14

ANTIGENIX AMERICA, INC.

PRODUCT INFORMATION SUMMARY

Human Apo-E3
ELISA Construction Kit

Product Number RHF335CK		Product Number **RHF335CKC**	
Approx. 960 tests		**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	100.0 ug	TMB Substrate (50 mL x 2)	
		Wash Buffer (20X)	100 mL

DESCRIPTION:
This ELISA CONSTRUCTION Kit provides antigen affinity purified capture and tracer antibodies, and antigen standard sufficient for **approximately** ten microplates.
Working concentrations must be optimized by customer.

Note: Reconstitute components only when ready to run assay.
Note: Some **X-reactivity** with human ApoE2 and human Apo-E4 observed.

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 of Biotin labeled, antigen-affinity purified antibody, additive-free. Reconstitute in 500 uL sterile water **containing 0.1% BSA**.

STANDARD: Provided as 100.0 ug of recombinant Human Apo-E3. Quick-spin and **reconstitute in** 100 uL of **20mM Sodium Phosphate (pH7.8) plus 0.5mM DTT** . Further dilutions can be made in 0.05% Tween-20, 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) 1.0 mL - store @ -20 Deg. C.
- TMB Substrate Solutions - Part A and Part B (50.0 mL each) cat # ES200C
- Wash Buffer (20X) Dilute 1 part with 19 mparts distilled water.

HANDLING/ STORAGE: Reconstitute reagents when ready to build ELISA assay. Antibodies (Capture and Tracer) can be stored for approximately one month at 4 Degrees C. Or store **frozen** at -20

Degrees C. for up to 6 months.

Standard (rec. Human ApoE3) can be stored in liquid state (@ 4 Deg. C.) For up to two days, or store **frozen, with addition of 0.1% BSA,** at -20 Deg. C. 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**
TMB Substrate (ANTIGENIX cat # **ES200**)
Dubelco's PBS (10X)
ANTIGENIX **ELISA Coating Stabilizer** (cat no: **EA150**)

RECOMMENDED SOLUTIONS:

See ANTIGENIX Developing Reagents above.

PBS: Dilute to 1XPBS in sterile water
WASH BUFFER: 0.05% Tween-20 in PBS.
BLOCK BUFFER: **use ANTIGENIX AMERICA coating stabilizer (EA150) or** 1% BSA in PBS
Substrate Solution: TMB Substrate Solution (ANTIGENIX # ES200)
Diluent: 0.05% Tween-20, 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 (cat # EA150)-recommended! -** 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 of plate- 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. With 0.1% BSA) from 20.0 ng/mL - 8 point, 1:2 or 1:3 serial dilution - (**Adjust range**- depending on desired range, and sensitivity of first standard curve) to zero in diluent. Immediately add 100 uL of standard or sample to each well in duplicate. Incubate at room temp. for approx. 2 hours.

DETECTION: Aspirate and wash plate 4 times. **Dilute** portion of detection (Tracer) antibody in diluent to concentration of 0.20 ug/mL. Add 100 uL per well. **Incubate** at room temperature for approx. **1 hour**. Note: detection antibody can be used in approximate range of 0.10 - 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 - 20 minutes by adding 100 uL of 2N Sulfuric acid to each well. Then, read plate @ 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 1.6 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.

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