PRODUCT INFORMATION SUMMARY

Human APRIL
ELISA Construction Kit

Product Number RHF921CK		Product Number **RHF921CKC** **With Developing Reagents**:	
Approx. 960 tests			
Capture Antibody	50.0 ug	ELISA Coating Stabilizer	50 mL
Biotin-Labeled tracer	25.0 ug	Streptavidin-HRP	1.0mL
Antigen Standard	2.0 ug	TMB Substrate (50 mL x 2)	

DESCRIPTION:
Human APRIL ELISA CONSTRUCTION Kit provides antigen affinity purified polyclonal capture and tracer antibodies, and antigen standard for development of **approximately** five to ten microplate assays. Working concentrations must be optimized by customer.
Note: Reconstitute components only when ready to run assay.

CAPTURE ANTIBODY:
Provided as lyophilized, 50 ug, additive-free. Reconstitute in 0.50 mL sterile water (0.1 mg/mL). (FREEZE aliquots for long-term storage)

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**. (FREEZE aliquots for long-term storage)

STANDARD: Provided as 2.0 ug of recombinant Human APRIL. Quick-spin and reconstitute in 50 uL of sterile water (pH 7.2). 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

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 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.

MATERIALS RECOMMENDED:

ELISA Microplates: Nunc Maxisorp, Prod. # 4420404
Tween -20.
BSA
Streptavidin-HRP: ANTIGENIX Cat no. **S100180** or similar
TMB Substrate : ANTIGENIX cat # **ES200** or similar
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 (cat # ES200)
Diluent: 0.05% Tween-20, 0.1% BSA in PBS
2N Sulfuric acid (stop solution).

PLATE PREPARATION:

Dilute **100 uL of** capture antibody with 0.05M Carbonate buffer (or PBS) to concentration 1.0 ug/mL. To optimize coating concentration on first test plate, use 2 test concentrations of capture antibody (for example: 1.0 ug/mL, and 0.5 ug/mL).

Immediately add 100 uL to each ELISA well. (With first test plate, coat one-half of plate with each concentration, and note which wells have different concentrations). Seal the plate and incubate overnight at room temperature.

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)** 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.

With coating stabilizer aspirate plate but **DO NOT WASH**. Dry plate in humidity controlled chamber or similar. (see data sheet EA150). If using standard block buffer, wash 4 times and tap plate dry.

PROTOCOL:

STANDARD/SAMPLE: Dilute **a portion of the** standard (store unused standard in aliquots, high concentration, frozen -20 Deg. C.) from 20.0 ng/mL (**Adjust,** depending on desired range and sensitivity of first standard curve) to zero in diluent (serial dilution). Immediately add 100 uL of standard or sample to each well in duplicate. Incubate at room temp. for appros 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 2 hours. 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 for substrate preparation and handling from manufacturer's data sheet). Incubate at room temp. for color development. Monitor color development with plate reader at 650 nm wavelength. (for blue color). The color reaction may be stopped after 10 – 30 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 0.2 units for the zero standard concentration, or 2.2 units for the highest standard concentration. Monitor the plate every 5 minutes for approximately 30 minutes.

WARRANTY:

Products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product.

NOTE: Kit can be ordered with the following developing reagents (suitable for approx. 1,000 wells – ten microplates) or order separately (larger sizes) as below:

ELISA Construction Kits

Accessory Reagents Available:

Streptavidin-HRP; **S100180**, 1.0 mL $ 190.00 USD ; suitable for 5,000 ELISA wells

TMB Substrate; **ES200**, 100 mL x 2, $95.00; suitable for 2,000 ELISA wells

ELISA Coating Stabilizer; **EA150**; $190.00, 100 mL (5X); suitable for 2,500 wells

Get All three reagents above as " **ELISA Construction Pack"** ; **EA700**; $410.00 USD.

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE