PRODUCT INFORMATION SUMMARY

Human GDNF
ELISA CONSTRUCTION KIT

Product Number RHF240CK		Product Number **RHF240CKC**	
Approx. 960 tests		**With Developing Reagents**:	
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 Glial-Derived Neurotrophic Factor (GDNF), This ELISA Construction kit supplies the main components to develop ELISA assay for human GDNF. Working concentrations of capture and tracer antibody are to 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 (100 ug/mL). (FREEZE aloquots 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 aloquots for long-term storage)

STANDARD: Provided as 2.0 ug of recombinant Human GDNF. Quick-spin and reconstitute in 50 uL of sterile water. 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. Reconstituted 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. NGF 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.

MATERIALS RECOMMENDED:

See ANTIGENIX Developing Reagents above.

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

RECOMMENDED SOLUTIONS:

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
Diluent: 0.05% Tween-20, 0.1% BSA in PBS
2N Sulfuric acid (stop solution).

PLATE PREPARATION:

1. 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.

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)** 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**. 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 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 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 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. 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 - 20 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.

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE

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.

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