

**PRODUCT INFORMATION SUMMARY****Human FGF-basic  
ELISA Construction Kit**

Product Number RHF322CK  
Approx. 960 tests

Product Number **RHF322CKC**  
**With Developing Reagents:**

Capture Antibody	100.0 ug	ELISA Coating Stabilizer	50 mL
Biotin-Labeled tracer	25.0 ug	Streptavidin-HRP	0.5 mL
Antigen Standard	10.0 ug	TMB Substrate ( 50 mL x 2)	
		Wash Buffer ( 20X)	

**RHF322CKP** -with Developing Reagents & 10 plates

**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.

**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 10.0 ug of recombinant Human FGF-basic. Quick-spin and **reconstitute in** 50 uL of **5mM Tris**, pH 7.6. Further dilutions can be made in diluent (see below).

**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) cat # ES200C
- 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 month at 4 Degrees C. Or store **frozen** at -20 Degrees C. for up to 6 months. Standard ( rec. FGF-basic) 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**  
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 cat # 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 - 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 plate 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 **3.0 ng/mL** to zero in diluent (1:2, 8-point serial dilution). 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 portion of detection (Biotin Tracer) antibody in diluent to concentration of 0.20 ug/mL. Add 100 uL per well. Incubate at room temperature for **1 hour**. Note: detection antibody can be used in approximate range of 0.10 - 0.40 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 (see data sheet ES200 for preparation of substrate solution). **Incubate** at room temp. for color development. **Stop** the color reaction after 10 - 15 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 0.30 units for the zero standard concentration, or 2.2 units for the highest standard concentration.

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

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