

## PRODUCT INFORMATION SUMMARY

### Human VEGF ELISA Construction Kit

Product Number RH88820CK  
Approx. 960 tests

Product Number **RH88820CKC**  
**With Developing Reagents:**

<b>Capture Antibody</b>	<b>50.0 ug</b>	ELISA Coating Stabilizer	50 mL
Biotin tracer	0.5 mL or 25.0 ug	Streptavidin-HRP	0.5mL
Antigen Standard	1.0 or 2.0 ug	TMB Substrate	( 50 mL x 2)
		Wash Buffer (20X)	100 mL

**RH88820CKP** w/ Developing Reagents & ten ELISA 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, **50.0 ug**, additive-free. Reconstitute in 0.50 mL sterile water (100.0 ug/mL).

#### TRACER ANTIBODY:

Provided as 25.0 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 1.0 ug or 2.0 ug ( **see vial**) of recombinant Human VEGF. 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 Stabilizer / Blocking Reagent ( EA150C) 50.0 mL ( 5X )
- 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 provided lyophilized - after reconstitution- (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.

For biotin tracer antibody provided as 0.5 mL liquid- STORE refrigerated only.

Standard ( rec. Human VEGF) 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**)  
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: ANTIGENIX WB200, or 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 **0.5 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 use of coating stabilizer -aspirate plate but **DO NOT WASH**. For extended storage- dry plate in humidity controlled

chamber or similar. ( see data sheet EA150). If using standard block buffer, then aspirate and WASH plate (4X). Tap plate dry.

#### **PROTOCOL:**

**STANDARD/SAMPLE:** Dilute **a portion of the** standard ( store unused standard in aliquots, high concentration, frozen -20 Deg. C.) from **2.0 ng/mL** to zero in diluent (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 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** after the 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 1.6 units for the highest standard concentration.

#### **X-Reactivity Data:**

X-Reactivity observed ( @ 40-50 ng/mL):

Mouse, Rat VEGF

**No** measurable X Reactivity observed with:

Human: VEGF-B, VEGF-C, VEGF-D, RANTES, GM-CSF, EGF, EG-VEGF, FGF-16, PDGF: forms -AA, AB, BB, CC,; SCF

Mouse: PDGF-AA, and -BB; EGF; SCF

Rat: EGF; SCF

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