

## ANTIGENIX AMERICA, INC.

### PRODUCT INFORMATION SUMMARY

#### Mouse VEGF Alpha ELISA Construction Kit

Product Number RMF433CK  
Approx. 960 tests

Product Number **RMF433CKC**  
**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	1.0 or 2.0 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.

#### CAPTURE ANTIBODY:

Provided as lyophilized, **100.0 ug**, additive-free. Reconstitute in 0.50 mL sterile water (200.0 ug/mL).

#### TRACER ANTIBODY:

Provided as 25 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 Mouse VEGF. 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) 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, 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.

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

Standard ( rec. Mouse 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 **W B200** 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 **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 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 **2.0 ng/mL** 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 the detection (Biotin 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 from manufacturer) Incubate at room temp. for color development. Monitor color development with plate reader at 650 nm wavelength ( 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 at 450 nm** ( correction set to 650 nm).

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.

## X-Reactivity Data:

X-Reactivity was observed @ 40-50 ng/mL with following factors:  
Rat VEGF and Human VEGF

No measurable X-Reactivity was observed with following:

Rat SCF

Mouse: SCF; GM-CSF; EGF; flt-3 ligand

Human: EG-VEGF; GM-CSF; SCF; EGF; flt-3 ligand; PDGF-AA, and AB and BB;

## REFERENCES:

CXCL12 /CXCR4 Blockade by Oncolytic Virotherapy Inhibits Ovarian Cancer Growth by Decreasing Immunosuppression...  
**J. Immunol. 2014; 193:5327-5337**