

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