

# ANTIGENIX AMERICA, INC.

## PRODUCT INFORMATION SUMMARY

### Human Visfatin ELISA Construction Kit

Product Number RHF783CK  
Approx. 960 tests

Product Number **RHF783CKC**  
**With Developing Reagents:**

Capture Antibody	<b>100.0 ug</b>	ELISA Coating Stabilizer	50 mL
Biotin-Labeled tracer	25.0 ug	Streptavidin-HRP	0.5mL
Antigen Standard	5.0 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). ( 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 5.0 ug of recombinant Human Visfatin. Quick-spin and reconstitute in 100 uL of sterile water. 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) 1.0 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. Visfatin) 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** (use 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 (recommended!) (EA150)**  
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 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, with addition of 0.1% BSA, frozen -20 Deg. C.) from **10.0 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 the 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 0.15 - 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. **1:1,500** 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. Monitor color development with plate reader at 650 nm wavelength ( blue color). The color reaction should be stopped after 10 - 20 minutes by adding 100 uL of 2N Sulfuric acid to each well. Then, **read plate @ 450 nm** after stop solution added.

NOTE: reliable standard curves are obtained when O.D. readings do not exceed 0.25 units for the zero standard concentration, or 2.0 units for the highest standard concentration.

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

NOTE: Kit can be ordered with developing reagents ( cat# ends in 'CKC') see page 1.

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