ANTIGENIX AMERICA Inc
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PRODUCT INFORMATION SUMMARY

Mouse EGF ELISA Construction Kit

Product Number RMF437CK Approx. 960 tests
Capture Antibody 50.0 ug
Biotin-Labeled tracer 25.0 ug
Antigen Standard 100.0 ug

DESCRIPTION:
This ELISA CONSTRUCTION Kit provides antigen affinity purified polyclonal 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).

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**.

STANDARD:
Provided as 100.0 ug of recombinant Mouse EGF. Quick-spin and reconstitute in 100 uL of sterile water. Further dilutions can be made in 0.05% Tween-20, 0.1% BSA in PBS.

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. Mouse EGF) can be stored in liquid state (@ 4 Deg. C.) For up to one week, or store **frozen** 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:
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 (ANTIGENIX # ES200)
Diluent: 0.05% Tween-20, 0.1% BSA in PBS

PLATE PREPARATION:
1.Dilute **100 uL of** capture antibody with 0.05M Carbonate buffer (or PBS) to concentration 1.0 ug/mL. To optimize coating concentration on first test plate, use 3 test concentrations of capture antibody (for example: 1.0 ug/mL, 0.5 ug/mL and 0.25 ug/mL). Immediately add 100 uL to each ELISA well. (With first test plate, coat one-third of plate with each concentration, and note which wells have different concentrations). 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 (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.Aspirate plate but **DO NOT WASH**. Dry plate in humidity controlled chamber or similar (see data sheet EA150).

PROTOCOL:
STANDARD/SAMPLE: Dilute **a portion of the** standard (store unused standard in aliquots, high concentration, frozen -20 Deg. C.) from 20.0 ng/mL or 40.0 ng/mL (depending on desired range) to zero in diluent (serial dilution). Immediately add 100 uL of standard or sample to each well in triplicate. Incubate at room temp. for at least 2 hours.
DETECTION: Aspirate and wash plate 4 times. Dilute detection (Tracer) antibody in diluent to concentration of 0.25 ug/mL. Add 100 uL per well. Incubate at room temperature for 2 hours. Note: detection antibody can be used in approximate range of 0.15 - 0.30 ug/mL, you may need to optimize for subsequent plates.
STREPTAVIDIN-HRP: Aspirate and wash plate 4 times. Dilute Streptavidin-HRP conjugate approx. 1:1000 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 405 nm wavelength. (correction set to 650 nm)
NOTE: reliable standard curves are obtained when O.D. readings do not exceed 0.2 units for the zero standard concentration, or 1.2 units for the highest standard concentration. Monitor the plate every 5 minutes for approximately 30 minutes.

WARRANTY:
Products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product.

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE