Mouse EGF ELISA Kit
(mEGF-ELISA)

Cat. No. EK0326

96 Tests in 8 x 12 divisible strips

Background

Epidermal growth factor (EGF) plays an important role in the regulation of cell growth, proliferation, and differentiation by binding to its receptor epidermal growth factor receptor (EGFR). Human EGF is a 6045-Da protein with 53 amino acid residues and three intramolecular disulfide bonds. EGF acts by binding with high affinity to EGFR on the cell surface and stimulating the intrinsic protein-tyrosine kinase activity of the receptor. EGF results in cellular proliferation, differentiation, and survival. It also has a profound effect on the differentiation of specific cells in vivo and is a potent mitogenic factor for a variety of cultured cells of both ectodermal and mesodermal origin. EGF has strong expression in kidney, salivary gland, cerebrum, and prostate; moderate expression in trachea and thyroid; and low expression in bone marrow, heart, spleen, thymus, uterus, and colon. No expression was detected in adrenal gland, liver, lung, cerebellum, placenta, and small intestine.

ScienCell's mouse EGF ELISA Kit is based on standard sandwich enzyme-linked immune-sorbent assay technology. Mouse EGF specific-monoclonal antibodies are precoated onto 8 x 12 divisible strips. The mouse specific detection polyclonal antibodies are biotinylated. The test samples and biotinylated detection antibodies are subsequently added to the wells and then washed with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex is added, and unbound conjugates re washed away with PBS or TBS buffer. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of yellow is proportional to the amount of mouse EGF that is captured in the strips.

Size 96 Tests in 8 x 12 divisible strips

Assay type Sandwich ELISA

Range 15.6 pg/ml-1000 pg/ml

Sensitivity < 1 pg/ml

Specificity No detectable cross-reactivity with other cytokine.

Storage Store at 4°C for frequent use, at -20°C for infrequent use.
Avoid multiple freeze-thaw cycles

Shipping Shipped on gel ice.

Expiration Four months at 4°C and eight months at -20°C.

Application For quantitative detection of mouse EGF in cell culture supernatants, serum, plasma (heparin, EDTA), tissue lysates and urine.

Kit components
1. Lyophilized recombinant mouse EGF standard: 10 ng/tube×2.
2. 8 x 12 divisible strips pre-coated with anti- mouse EGF antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- mouse EGF antibody: 130µl, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC): 130µl, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.
9. TMB stop solution: 10ml.

Materials Required But Not Provided
1. Microplate reader.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multi-channel pipettes are recommended for large number of samples.
4. Clean tubes and Eppendorf tubes.
5. Washing buffer (neutral PBS or TBS).
   Preparation of 0.01M TBS: Add 1.2g Tris, 8.5g Nacl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.
   Preparation of 0.01 M PBS: Add 8.5g sodium chloride, 1.4g Na_2HPO_4 and 0.2g NaH_2PO_4 to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Usage This product is for research use only. It is not approved for use in humans, animals, or *in vitro* diagnostic procedures.

Reference

1. Herbst RS (2004). "Review of epidermal growth factor receptor biology". International Journal of Radiation Oncology, Biology, Physics 59 (2 Suppl): 21–6.
2. Carpenter, G.; Cohen, S.: Epidermal growth factor. Ann. Rev. Biochem. 48: 193-216, 1979.
3. Groenestege, W. M. T.; Thebault, S.; van der Wijst, J.; van den Berg, D.; Janssen, R.; Tejpar, S.; van den Heuvel, L. P.; van Cutsem, E.; Hoenderop, J. G.; Knoers, N. V.; Bindels, R. J. : Impaired basolateral sorting of pro-EGF causes isolated recessive renal hypomagnesemia. J. Clin. Invest. 117: 2260-2267, 2007.

Protocol for Mouse EGF ELISA (96 well format)

Notes before you begin

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
2. The TMB Color developing agent should be colorless and transparent before using.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. A duplicate well assay is recommended for both standard and samples.
5. Do not let wells dry, as this will inactivate active components in wells.

6. Do not reuse tips and tubes to avoid cross contamination.
7. Avoid using reagents from different batches.
8. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution be pre-warmed in 37°C for 30 minutes before use.

Preparation

Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- **Cell culture supernatants or tissue lysates**: Remove particulates by centrifugation, assay immediately or aliquot and store at -20°C.
- **Serum**: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store samples at -20°C.
- **Plasma**: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1500 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C.
- **Urine**: Aseptically collect the first urine of the day; micturate directly into a sterile container. Remove particular impurities by centrifugation, assay immediately or aliquot and store samples at -20°C.

Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be well mixed with the diluent buffer**.

- **High target protein concentration (10-100 ng/ml)**. The working dilution is 1:100. i.e. Add 1 μl sample into 99 μl sample diluent buffer.
- **Medium target protein concentration (1-10 ng/ml)**. The working dilution is 1:10. i.e. Add 10 μl sample into 90 μl sample diluent buffer.
- **Low target protein concentration (15.6-1000 pg/ml)**. The working dilution is 1:2. i.e. Add 50 μl sample to 50 μl sample diluent buffer.
- **Very Low target protein concentration (≤ 15.6 pg/ml)**. No dilution necessary, or the working dilution is 1:2.

Reagent Preparation and Storage

A. Reconstitution of the mouse EGF standard: EGF standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of EGF standard (10 ng per tube) are included in each kit. Use one tube for each experiment.

- 10000pg/ml of mouse EGF standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 minutes and mix thoroughly.
- 1000pg/ml of mouse EGF standard solution: Add 0.1 ml of the above 10ng/ml EGF standard solution into 0.9 ml sample diluent buffer and mix thoroughly.
- 500pg/ml→15.6pg/ml of mouse EGF standard solutions: Label 6 Eppendorf tubes with 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.3pg/ml, 15.6pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 1000pg/ml EGF standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 10 ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti-mouse EGF antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

- The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
- Biotinylated anti-mouse EGF antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.

- The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
- Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly.

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard EGF detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of EGF amount in samples.

1. Aliquot 0.1ml per well of the 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.3pg/ml, 15.6pg/ml mouse EGF standard solutions into the precoated wells Add 0.1 ml of the sample diluent buffer into the control well (**blank well**). Add 0.1ml of each properly diluted sample of mouse cell culture supernatants, serum, plasma (heparin, EDTA), tissue lysates or urine to each empty well. See "**Sample Dilution Guideline**" above for details. We recommend that each mouse EGF standard solution and each sample is measured in duplicate.
2. Seal the strips with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard strips' contents, and blot the strips onto paper towels or other absorbent material. **Do NOT** let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-mouse EGF antibody working solution into each well and incubate the strips at 37°C for 60 min.
5. Wash strips 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the strips onto paper towels or other absorbent material. (**Strips Washing Method**: Discard the solution in the strips without touching the side walls. Blot the strips onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the strips onto paper towels or other absorbent material).
6. Add 0.1ml of prepared ABC working solution into each well and incubate the strips at 37°C for 30 min.
7. Wash strips 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the strips onto paper towels or other absorbent material.(See Step 5 for strips washing method).
8. Add 90 μl of prepared TMB color developing agent into each well and incubate strips at 37°C in dark for 20-25 minutes (**Note**: For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated mouse EGF standard solutions; the other wells show no obvious color).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The mouse EGF concentration of the samples can be interpolated from the standard curve.
Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Summary

1. Add samples and standards and incubate the strips at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the strips at 37°C for 60 min. Wash strips 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the strips at 37°C for 30 min. Wash strips 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the strips at 37°C in dark for 20-25 min.
5. Add TMB stop solution and read.

Typical Data Obtained from Mouse EGF

(TMB reaction incubate at 37°C for 22 min)

Concentration (pg/ml)	0.0	15.6	31.3	62.5	125	250	500	1000
Absorbance (450 nm)	0.035	0.189	0.284	0.462	0.852	1.223	1.629	2.145

Typical Mouse EGF ELISA Kit Standard Curve

This standard curve was generated for demonstration purpose only. A standard curve must be run with each assay.