MULTI SCIENCES

Human EGF ELISA Kit

【Catalog No】EK193
【SIZE】48T/96T
【INTENDED USE】For the quantitative determination of human Epidermal Growth Factor (EGF) concentrations in cell culture supernates, serum and plasma.

【INTRODUCTION】

Epidermal growth factor (EGF) is a growth factor that stimulates cell growth, proliferation, and differentiation by binding to its receptor EGFR. EGF is a low-molecular-weight polypeptide first purified from the mouse submandibular gland, but since then found in many human tissues including submandibular gland, parotid gland. Salivary EGF, which seems also regulated by dietary inorganic iodine, also plays an important physiological role in the maintenance of oro-esophageal and gastric tissue integrity. The biological effects of salivary EGF include healing of oral and gastroesophageal ulcers, inhibition of gastric acid secretion, stimulation of DNA synthesis as well as mucosal protection from intraluminal injurious factors such as gastric acid, bile acids, pepsin, and trypsin and to physical, chemical and bacterial agents. Increased activity of the receptor for EGF has been observed in certain types of cancer, often correlated with mutations in the receptor and abnormal function such as constitutive receptor signalling independent of the levels of EGF or of binding of EGF.

【PRINCIPLE OF THE ASSAY】

Human EGF ELISA Kit is based on the quantitative sandwich enzyme-linked immunosorbent assay technique to measure concentration of human EGF in the samples. A monoclonal antibody specific for human EGF has been immobilized onto microwells. Standard or samples are pipetted into the wells, followed by the addition of biotin-linked detect antibody specific for EGF, and EGF present is bound by the immobilized antibody and detect antibody following the first incubation. After removal of any unbound substances, streptavidin-HRP is added for a second incubation. After washing, substrate solution reacts with HRP and color develops in proportion to the amount of EGF bound by the immobilized antibody. The color development is stopped by addition of acid and the optical density value is measured by microplate reader.

【MATERIALS PROVIDED】

PART	PART #	EK193-48	EK193-96
Coated Microplate	EK193P	48T	96T
Standard	EK193S	1 vial	2 vials
Detect antibody	EK193D	1 vial	1 vial
Standard Diluent	E0260	5ml	5ml
Streptavidin-HRP	E0290	1 vial	1 vial
Assay Buffer (10×)	E0310	5ml	5ml
TMB	E0230	6ml	11ml
Stop Solution	E0300	11ml	11ml
Washing Buffer (20×)	E0281	50ml	50ml
Adhesive Film	E0200	6	6

Note: Components from reagent kits of different batch numbers must not be used interchangeably.

OTHER SUPPLIES REQUIRED

1) Microplate reader capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.
2) Pipettes and pipette tips.
3) 50 μl to 300 μl adjustable multichannel micropipette with disposable tips.
4) Multichannel micropipette reservoir.

5) Beakers, flasks, cylinders necessary for preparation of reagents.
6) Deionized or distilled water.
7) Polypropylene test tubes for dilution.

【STORAGE】

Store at 2-8℃; refer to the kit label for expiration date.
For opened kits:
Pre-coated microplate: Can be stored at 2-8℃ for approximately 1 month (return unused strips to the aluminum foil bag and reseal).
Standard: Can be stored at -20℃ for approximately 1 month (discard after single-use reconstitution).
Other components: Can be stored at 2-8℃ for approximately 1 month.

【SAMPLE COLLECTION AND STORAGE】

1) **Cell Culture Supernates** – Remove particulates by centrifugation and assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.
2) **Serum** – Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1,000 × g. Remove serum and assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.
3) **Plasma** – Collect plasma using EDTA, citrate or heparin as anticoagulant. Centrifuge at 1,000 × g within 30 minutes of collection. Assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.
4) might be suitable for use in the assay. Cell culture supernates, serum and plasma were tested with this assay. Dilution with Assay Buffer may be needed.
Note: Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.
If samples are to be run within 24 hours, they may be stored at 2 to 8℃. For longer storage, aliquot samples and store frozen at -20℃. Avoid repeated freeze-thaw cycles.

【REAGENT PREPARATION】

Bring all reagents and samples to room temperature before use.
If crystals form in the Buffer Concentrates, warm and gently stir them until completely dissolved.

Washing Buffer (1×)

Pour entire contents (50 ml) of the **Washing Buffer (20×)** into a clean 1,000 ml graduated cylinder. Bring to final volume of 1,000 ml with pure or deionized water.
Mix gently to avoid foaming.
Transfer to a clean wash bottle and store at 2 to 25℃. Washing Buffer (1×) is stable for 30 days.

Assay Buffer (1×)

Pour the entire contents (5 ml) of the **Assay Buffer (10×)** into a clean 100 ml graduated cylinder. Bring to final volume of 50 ml with distilled water. Mix gently to avoid foaming.
Store at 2 to 8℃. Assay Buffer (1×) is stable for 30 days.

Detect Antibody

Mix well prior to making dilutions.
Make a **1：100** dilution of the concentrated **Detect Antibody** solution with Assay Buffer (1×) in a clean plastic tube.
The diluted Detect Antibody should be used within 30 minutes after dilution.

Streptavidin-HRP

Mix well prior to making dilutions.
Make a **1：100** dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1×) in a clean plastic tube as needed.

The diluted Streptavidin-HRP should be used within 30 minutes after dilution.

Sample Dilution: If your samples have high **EGF** content, dilute serum/plasma samples with Assay Buffer (1×). For cell culture supernates, dilute with cell culture medium.

Human EGF Standard: Reconstitute Human EGF Standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 1,000 pg/ml). Allow the standard to reconstitute for 10 - 30 minutes. Mix well prior to making dilutions.

Use polypropylene tubes.

● **For serum/plasma samples**, mixing concentrated human EGF standard (230 µl) with 230 µl of Standard Diluent creates the high standard (500 pg/ml). Pipette 230 µl of Standard Diluent into each tube. Use the high standard to produce a 1:1 dilution series (scheme below). Mix each tube thoroughly before the next transfer. Standard Diluent serves as the zero standard (0 pg/ml).

● **For cell culture supernates**, mixing concentrated human EGF standard (230 µl) with 230 µl of cell culture medium creates the high standard (500 pg/ml). Pipette 230 µl of cell culture medium into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. Cell culture medium serves as the zero standard (0 pg/ml).
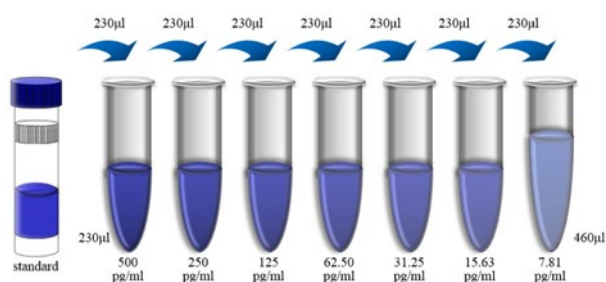
【ASSAY PROCEDURE】

Bring all reagents and samples to room temperature before use.

1) Prepare all reagents including microplate, samples, standards and working solution as described in the previous sections.

2) Remove excess microplate strips and return them to the foil pouch containing the desiccant pack, and reseal for further use.

3) Add 300 µl Washing Buffer (1×) per well, and allow it for about 30 seconds before aspiration. Soaking is highly recommended to obtain a good test performance. Empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Washing Buffer (1×). Use the microwell strips immediately after washing. **Do not allow wells to dry.**

4) Add 100 µl of 2-fold diluted Standard in duplicated. Add 100 µl of Standard Diluent/culture medium to Blank well in duplicate.

5) **Serum/Plasma:** Add 80 µl of Assay Buffer (1×) and 20 µl sample to the sample well. **Cell culture supernates:** Add 100 µl cell culture supernates to the sample well.

6) Add 50 µl of diluted Detect Antibody to each well. Ensure reagent addition in step 4, 5 and 6 is uninterrupted and completed within 15 minutes.

7) **Seal the plate with an adhesive film.** Incubate at room temperature (25℃±3℃) for 2 hours on a microplate shaker set at 300 rpm.

8) Aspirate each well and wash by filling each well with 300 µl Washing Buffer (1×), repeat five times for a total six washes. Complete removal of liquid at each step is essential to the best performance. After the last wash, remove any remaining Washing Buffer (1×) by aspirating or decanting. Invert the plate and tap it against clean paper towels.

9) Add 100 µl of diluted Streptavidin-HRP to each well.

10) Seal the plate with a fresh adhesive film. Incubate at room temperature (25℃±3℃) for 45 minutes on a microplate shaker set at 300 rpm.

11) Repeat aspiration/wash as in step 8.

12) Add 100 µl of Substrate Solution to each well. Incubate for 5 - 30 minutes at room temperature. **Protect from light.**
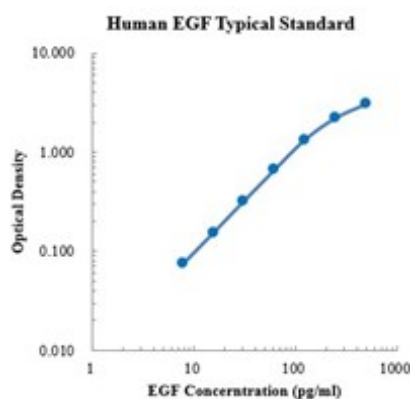
13) Add 100 µl of Stop Solution to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

14) Measure the optical density value within 30 minutes by microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Reading directly at 450 nm without correction may generate higher concentration than true value.

【TYPICAL DATA】

A standard curve must be run within each assay. The following standard curve is provided for demonstration only.

pg/ml	O.D.		Average	Corrected
0.00	0.027	0.023	0.025	
7.81	0.099	0.098	0.099	0.074
15.63	0.178	0.177	0.178	0.153
31.25	0.341	0.348	0.345	0.320
62.50	0.697	0.673	0.685	0.660
125.00	1.368	1.340	1.354	1.329
250.00	2.232	2.191	2.212	2.187
500.00	3.089	3.008	3.049	3.024

Human EGF Typical Standard