HUMAN EGF ELISA KIT

E16HE0044

Introductions

Epidermal growth factor (EGF) is the founding member of the EGF family that also includes TGFα, amphiregulin (AR), betacellulin (BTC), epiregulin(EPR), heparinbinding EGF-like growth factor (HB-EGF), epigen, and the neuregulins (NRG)1 through-6. Members of the EGF family share a structural motif, the EGF-like domain, which is characterized by three intramolecular disulfide bonds that are formed by six similarly spaced conserved cysteine residues. All EGF family members are synthesized as type I transmembrane precursor proteins that may contain several EGF domains in the extracellular region. The 1207 amino acid (aa) human EGF precursor contains nine EGF domains and nine LDLR class B repeats. The mature protein consists of 53 aa and is generated by proteolytic excision of the EGF domain proximal to the transmembrane region. EGF is present in various body fluids, including blood, milk, urine, saliva, seminal fluid, pancreatic juice, cerebrospinal fluid, and amniotic fluid. Four ErbB (HER) family receptor tyrosine kinases including EGFR/ErbB1, ErbB2, ErbB3 and ErbB4, mediate responses to EGF family members. These receptors undergo a complex pattern of ligand induced homo- or hetero-dimerization to transducer EGF family signals. EGF binds ErbB1 and depending on the context, induces the formation of homodimers or heterodimers containing ErbB2. Dimerization results in autophosphorylation of the receptor at specific tyrosine residues to create docking sites for a variety of signaling molecules. Biological activities ascribed to EGF include epithelial development, angiogenesis, inhibition of gastric acid secretion, fibroblast proliferation, and colony formation of epidermal cells in culture.

Principle of the Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for EGF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any EGF present is bound by the immobilized antibody. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for EGF is added to the wells and binds to the combination of capture antibody- EGF in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A coloured product is formed in proportion to the amount of EGF present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven EGF standard dilutions and EGF sample concentration determined.

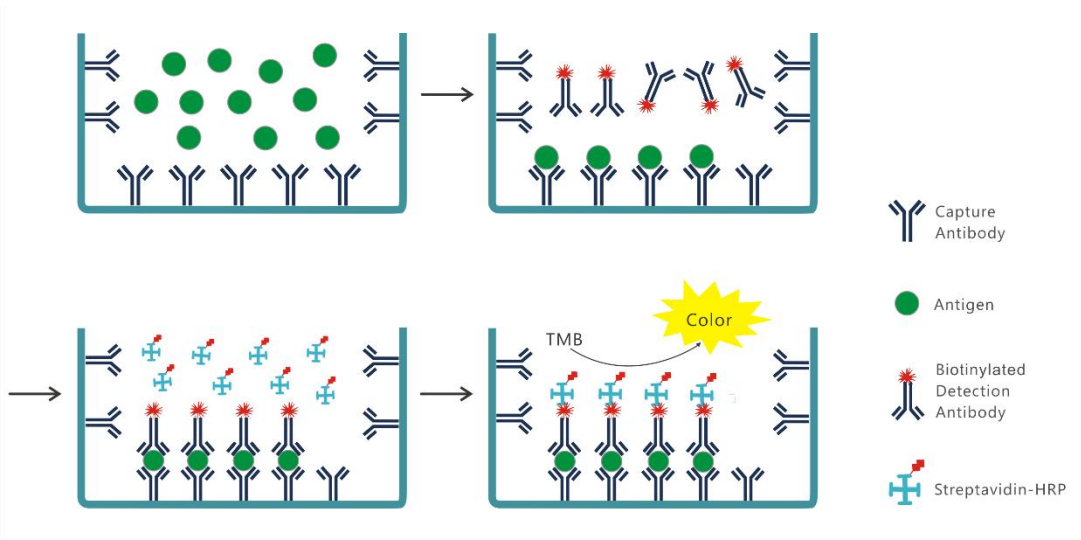


Figure 1. Schematic diagram of the assay

Materials supplied

Table 1. Kit Components

Kit Components	**96 wells Quantity/Size**
Aluminium pouches with a Microwell Plate coated with monoclonal antibody to human EGF (8 * 12)	1 plate
Human EGF Standard lyophilized, 20 ng/ml upon reconstitution	2 vials
Concentrated Biotin-Conjugate anti-human EGF monoclonal antibody	2 vials
Streptavidin-HRP solution	2 vials
Standard /sample Diluent	1 bottle
Biotin-Conjugate antibody Diluent	1 bottle
Streptavidin-HRP Diluent	1 bottle
Wash Buffer Concentrate 20x (PBS with 1% Tween-20)	1 bottle
Substrate Solution	1 vial
Stop Solution	1 vial
Adhesive Films	4 pieces
Product data sheet	1 copy

Storage

Table 2. Storage of the kit

Unopened Kit	Store at 2 - 8°C. Do **NOT** use past kit expiration date**!**	
Opened/ Reconstituted Reagents	Standard /Sample Diluent	May be stored for up to 1 month at 2 - 8°C.**
	Concentrated Biotin-Conjugate	
	Streptavidin-HRP Solution	
	Biotin-Conjugate Antibody Diluent	
	Streptavidin-HRP Diluent	
	Wash Buffer Concentrate 20x	
	Substrate Solution	
	Stop Solution	
	Standard	Aliquot and store for up to 1 month at ≤20°C. Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8°C.**

**Provided this is within the expiration date of the kit.

Materials Needed but Not Supplied

1. Microplate reader (450nm).
2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000ul.
3. 37℃ incubator.
4. Double-distilled water or deionized water.
5. Coordinate paper.
6. Graduated cylinder.

Precautions for Use

1. Store kit reagents between 2℃and 8℃. After use all reagents should be immediately returned to cold storage (2℃ to 8℃).
2. Please perform simple centrifugation to collect the liquid before use.
3. To avoid cross contamination, please use disposable pipette tips.
4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.
5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents and samples well before use.
6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.
7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.
8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.
10. Avoid microtiter plates drying during the operation.
11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.
13. This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

Sample Collection and Storage

1. **Cell Culture Supernates** - Remove particulates by centrifugation.
2. **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.

3. **Plasma** - Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.

4. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.

5. Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor).
   Note: The normal human serum or plasma samples are suggested to make a 1:2 dilution.

Reagent Preparation

1. Bring all reagents to room temperature before use.

2. **Wash Buffer** - Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard** - Reconstitute the Standard with 1.0mL of Standard /sample Diluent. This reconstitution produces a stock solution of 20000 pg /mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

   Pipette 900µL of Standard/sample Diluent into the 2000 pg/mL tube and pipette 500µL of Standard/sample Diluent into the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 2000 pg/mL standard serves as the high standard. The Standard/ sample Diluent serves as the zero standard (0 pg/mL).

   If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.

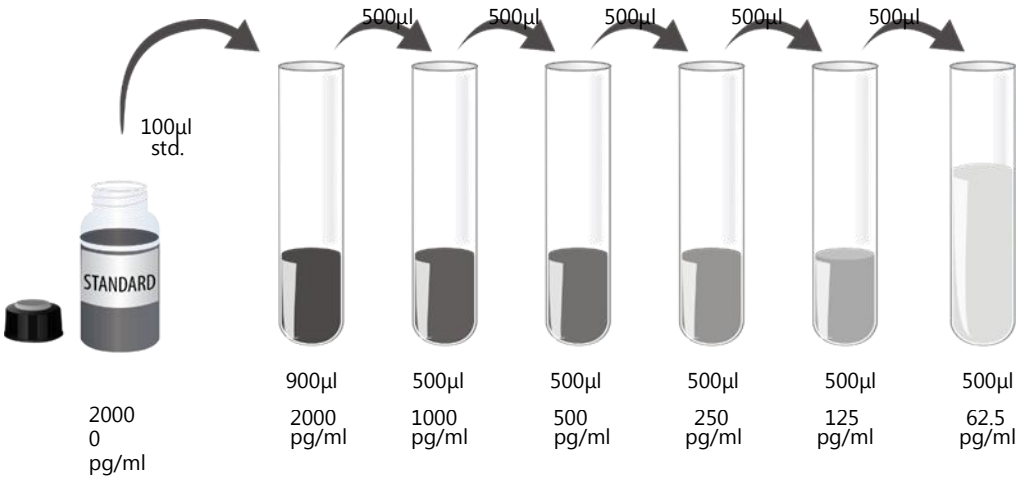


Figure 2. Preparation of EGF standard dilutions

4. Working solution of Biotin-Conjugate anti-human EGF monoclonal antibody:
   Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

   The working solution should be used within one day after dilution.

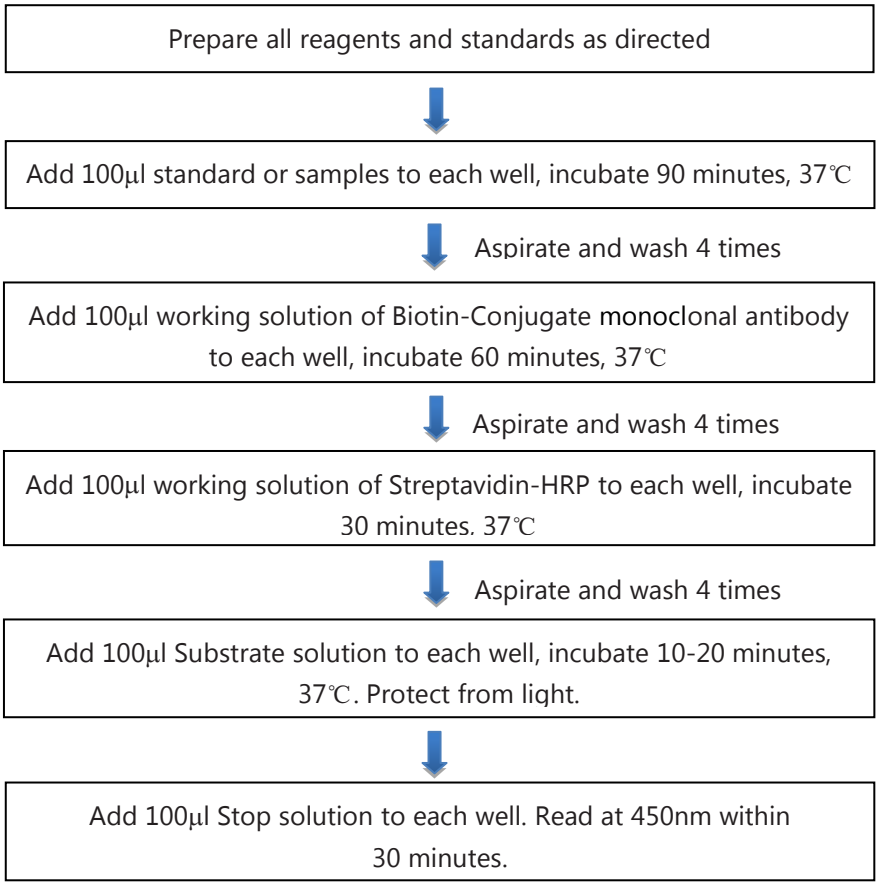
5. Working solution of Streptavidin-HRP: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

   The working solution should be used within one day after dilution.

General ELISA Protocol

1. Prepare all reagents and working standards as directed in the previous sections.
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8℃ sealed tightly.
3. Add 100μL of Standard, control, or sample, per well. Cover with the adhesive strip provided. Incubate for 1.5 hours at 37℃.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350μL) using a squirt bottle, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 μL of the working solution of Biotin-Conjugate to each well. Cover with a new adhesive strip and incubate 1 hours at 37℃.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 μL of the working solution of Streptavidin-HRP to each well. Cover with a new adhesive strip and incubate for 30 minutes at 37℃. Avoid placing the plate in direct light.
8. Repeat the aspiration/wash as in step 4.
9. Add 100 μL of Substrate Solution to each well. Incubate for 10-20 minutes at 37℃. Avoid placing the plate in direct light.
10. Add 100 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. (optionally 630nm as the reference wave length; 610-650nm is acceptable)

Assay Procedure Summary



Technical Hints

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.

5. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

Calculation of Results

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.
2. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
3. The data may be linearized by plotting the log of the EGF concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
4. This standard curve below is provided ***for demonstration only***. A standard curve should be generated for each set of samples assayed.

Table 3. Typical data using the EGF ELISA (Measuring wavelength: 450nm, Reference wavelength: 630nm)

Standard (pg/ml)	OD.	OD.	Average	Corrected
0	0.019	0.016	0.018	——
31.25	0.068	0.058	0.063	0.045
62.5	0.130	0.136	0.133	0.115
125	0.231	0.225	0.228	0.210
250	0.417	0.420	0.419	0.401
500	0.803	0.812	0.808	0.862
1000	1.425	1.430	1.428	1.410
2000	2.095	2.089	2.092	2.074

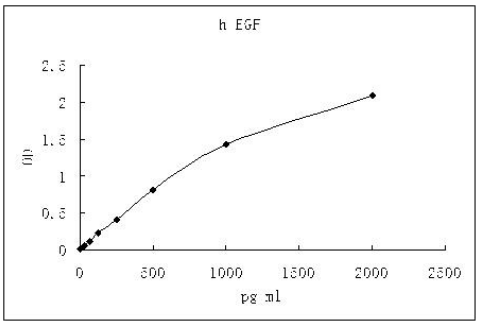


Figure 4. Representative standard curve for human EGF ELISA.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

Performance Characteristics

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

SENSITIVITY: The minimum detectable dose was 4pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant human EGF. The factors listed below were prepared at 50ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Table 4. Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Other proteins
G-CSF	IL-1β	bovine FGF acidic
GM-CSF	IL-3	bovine FGF basic
IL-1α	IL-4	human PDGF
IL-1β	IL-5	porcine PDGF
IL-2	IL-6	porcine TGF-β1
IL-3	IL-7	porcine TGF-β2
IL-4	EGF	
IL-6	GM-CSF	
IL-7	Pro-EGF	
IL-8		
LIF		
TGF-α		
TGF-β1		
TNF-α		
TNF-β		

References:

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