



Human EGF ELISA Kit

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Hinweis/Note:

Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

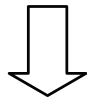
The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

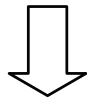
Thank you for choosing Assaypro.

Assay Summary

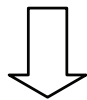
Add 50 μ l of Standard/ Sample per well.
Incubate 2 hours.



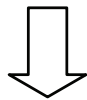
Wash, then add 50 μ l of
Biotinylated Antibody per well.
Incubate 2 hours.



Wash, then add 50 μ l of
SP Conjugate per well.
Incubate 30 minutes.



Wash, then add 50 μ l of
Chromogen Substrate per well.
Incubate 12 minutes.



Add 50 μ l of Stop Solution per well.
Read at 450 nm immediately.

AssayMax Human Epidermal Growth Factor (EGF) ELISA Kit

Catalog No. EE2011-1
Sample Insert/Reference Only

Introduction

Human epidermal growth factor (EGF) is a mitogenic growth factor that plays important roles in cell growth, proliferation, and differentiation. EGF is synthesized as a large precursor (1207 amino acids, 134 kDa) that is cleaved into a small mature protein (53 amino acids, 6 kDa). The precursor has 66% identity with the corresponding mouse protein (1-3). Its gene mutation causes autosomal recessive renal hypomagnesemia (4). EGF binds to the cell surface receptor EGFR, leading to the phosphorylation of the receptor tyrosine kinase and subsequent activation of the signal transduction pathway. The EGFR inhibition by small molecule tyrosine kinase inhibitors and monoclonal antibodies is the target of non-small cell lung cancer, colorectal cancer, pancreatic cancer, and breast cancer therapies (5-7).

Principle of the Assay

The AssayMax Human Epidermal Growth Factor (EGF) ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human EGF in urine, saliva, milk, and cell culture supernatant samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human EGF in less than 5 hours. A polyclonal antibody specific for human EGF has been pre-coated onto a 96-well microplate with removable strips. EGF in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for EGF, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**

- **Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

Reagents

- **Human EGF Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human EGF.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human EGF Standard:** Human EGF in a buffered protein base (2 ng, lyophilized).
- **Biotinylated Human EGF Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against EGF (140 μ l).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 μ l).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard at 2-8°C before reconstituting with diluent and at -20°C after reconstituting with diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.

- Pipettes (1-20 μl , 20-200 μl , 200-1000 μl , and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation and Storage

- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20°C or below. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. Dilute urine 1:60 with MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 800 x *g* for 10 minutes and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute milk 1:20 with MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 2 ng of Human EGF Standard with 2 ml of MIX Diluent to generate a 1 ng/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (1 ng/ml) 1:2 with MIX Diluent to produce 0.5, 0.25, 0.125, 0.063, 0.031, and 0.016 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[EGF] (ng/ml)
P1	Standard (1 ng/ml)	1.000
P2	1 part P1 + 1 part MIX Diluent	0.500
P3	1 part P2 + 1 part MIX Diluent	0.250
P4	1 part P3 + 1 part MIX Diluent	0.125
P5	1 part P4 + 1 part MIX Diluent	0.063
P6	1 part P5 + 1 part MIX Diluent	0.031
P7	1 part P6 + 1 part MIX Diluent	0.016
P8	MIX Diluent	0.000

- **Biotinylated Human EGF Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, working standards, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Human EGF Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Human EGF Antibody to each well and incubate for 2 hours.
- Wash the microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.

- Add 50 μ l of Chromogen Substrate per well and incubate for about 12 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μ l of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

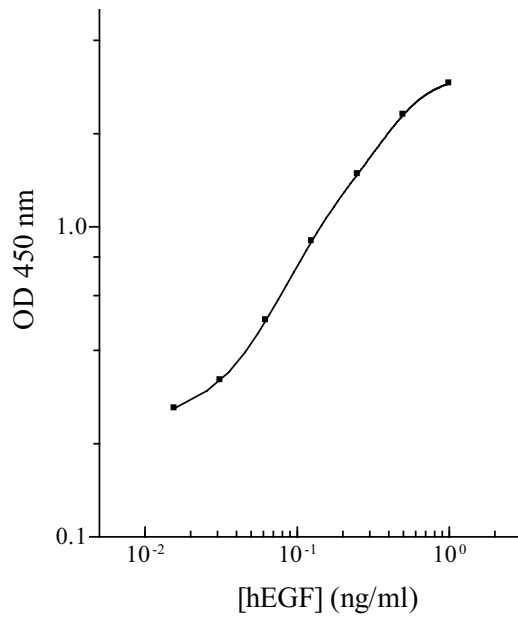
Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using four-parameter or log-log logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

Human EGF Standard Curve



Sensitivity and Specificity

- The minimum detectable dose of EGF is typically ~ 0.01 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9% and 7.2% respectively.

Linearity

Sample Dilution	Saliva
No Dilution	89%
1:2	96%
1:4	105%

Sample Dilution	Urine
1:30	92%
1:60	97%
1:120	104%

Sample Dilution	Milk
1:10	92%
1:20	98%
1:40	104%

Recovery

Standard Added Value	0.03 – 0.5 ng/ml
Recovery %	84 – 112%
Average Recovery %	97%

Cross-Reactivity

Species	% Cross Reactivity
Canine	10%
Bovine	None
Monkey	5%
Mouse	None
Rat	None
Rabbit	None
Human	100%

References

- (1) Carpenter G (1981) *Handbook of Experimental Pharmacology* 57:89-132
- (2) Taylor JM *et al.* (1972) *J. Biol. Chem.* 247:5928-5934
- (3) Bell GI *et al.* (1986) *Nucleic Acids Res.* 14(21):8427-8446
- (4) Groenestege WM *et al.* (2007) *J Clin Invest.* 117(8):2260-2267
- (5) Heist RS and Christiani D (2009) *Pharmacogenomics.* 10(1): 59–68
- (6) Markman B *et al.* (2010) *Adv Clin Chem.* 51:71-119
- (7) Renouf D and Moore M. (2010) *Expert Rev Anticancer Ther.* 10(4):529-540

Version 1.4