ELISA Kit
Catalog #KHG0061 (96 tests)
#KHG0062 (192 tests)

Human

EGF

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PURPOSE

The Invitrogen Human Epidermal Growth Factor (Hu EGF) ELISA is to be used for the quantitative determination of Hu EGF in human serum, plasma, buffered solution, cell culture medium or urine. The assay will recognize both natural and recombinant Hu EGF.

INTRODUCTION

Epidermal Growth Factor (EGF) is a 53-amino acid polypeptide originally discovered in the mouse submaxillary gland as an agent that induced precocious eyelid opening and early incisor eruption in the newborn mouse. EGF has been subsequently shown to elicit an array of biological responses that are mediated by specific binding to an EGF receptor/tyrosine kinase located on the cell surface. EGF induces cell proliferation, regulates tissue differentiation, modulates organogenesis, promotes angiogenesis, and accelerates wound healing. It also acts as an inhibitor of gastric acid secretion.

EGF is a member of a family of EGF-related growth factors including TGFα, heparin binding EGF-like growth factor (HB-EGF), epiregulin, amphiregulin (AR), betracellulin (BTC), neuregulin 1, neuregulin 2, and neuregulin 3. EGF is initially synthesized as a precursor of a 160-170 kDa glycoprotein. The mature and soluble form of EGF is a ~6 kDa protein which can be detected in many tissues and nearly all body fluids. High levels of EGF exist in some body fluids such as the saliva, urine, gastric juice, and mammary and seminal fluids.

EGF, triggers an intracellular signaling cascade through binding to EGF receptor (also known as ErbB1), causing changes within the target cells.

The EGF receptor, a receptor tyrosine kinase, upon stimulation by EGF results in the phosphorylation of its distinct tyrosine residues and phosphorylates the adaptor protein, SHC. Subsequently, the complex activates the Ras/mitogen activated protein (MAP) kinase pathway. This cascade couples EGF stimulation to gene transcription.

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Read entire protocol before use.

PRINCIPLE OF THE METHOD

The Invitrogen human EGF kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A polyclonal antibody specific for human EGF has been coated onto the wells of the microtiter strips provided. Samples, including standards of known human EGF content, control specimens, and unknowns, are pipetted into these wells.

During the first incubation, the Hu EGF antigen binds to the immobilized (capture) antibody on one site. After washing, a biotinylated monoclonal antibody specific for Hu EGF is added. During the second incubation, this antibody binds to the immobilized Hu EGF captured during the first incubation.

After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a third incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Hu EGF present in the original specimen.

REAGENTS PROVIDED

Note: *Store all reagents at 2 to 8°C.*

Reagent	***96 Test Kit***	***192 Test Kit***
Hu EGF Standard, purified recombinant Hu EGF expressed in *E. coli*. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials
Standard Diluent Buffer. Contains 0.1% sodium azide; 25 mL per bottle.	1 bottle	2 bottles
Antibody Coated Wells, 12x8 Well Strips	1 plate	2 plates
Hu EGF Biotin Conjugate (Biotin-labeled anti-EGF). Contains 0.1% sodium azide; 11 mL per bottle.	1 bottle	2 bottles
Streptavidin-HRP (100X). Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial	2 vials
Streptavidin-HRP Diluent. Contains 3.3 mM thymol; 25 mL per bottle.	1 bottle	1 bottle
Wash Buffer Concentrate (25X); 100 mL per bottle.	1 bottle	1 bottle
Stabilized Chromogen, Tetramethylbenzidine (TMB); 25 mL per bottle.	1 bottle	1 bottle
Stop Solution; 25 mL per bottle.	1 bottle	1 bottle
Plate Covers, adhesive strips.	3	6

SUPPLIES REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Distilled or deionized water.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semilog, as desired.
6. Glass or plastic tubes for diluting and aliquoting standard.
7. Absorbent paper towels.
8. Calibrated beakers and graduated cylinders in various sizes.

PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.

5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis. **Since EGF is present in platelets, and is released during activation, platelet-poor plasma should be used when quantitation of circulating levels of EGF is desired.**
6. It is recommended that all standards, controls and samples be run in duplicate.
7. Samples that are >250 pg/mL should be diluted with *Standard Diluent Buffer*.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. Cover or cap all reagents when not in use.
10. **Do not mix or interchange different reagent lots from various kit lots.**
11. Read absorbances within 2 hours of assay completion.
12. Do not use reagents after the kit expiration date.
13. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
14. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
15. Because *Stabilized Chromogen* is light sensitive, avoid prolonged exposure to light. Also avoid contact between *Stabilized Chromogen* and metal, or color may develop.

SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with *Wash Buffer Concentrate (25X)* provided.

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under **ASSAY METHOD**. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

If using an automated washer, the operating instructions for washing equipment should be carefully followed. If your automated washer allows, 30 second soak cycles should be programmed into the wash cycle.

REAGENT PREPARATION AND STORAGE

One microgram of the Invitrogen recombinant human EGF equals 1046 International Units of WHO reference preparation 91/530 (NIBSC, Hertfordshire, UK, EN6 3QG).

A. Reconstitution and Dilution of Human EGF Standard

Note: Either glass or plastic tubes may be used for standard dilutions.

1. Reconstitute standard to 5000 pg/mL with *Standard Diluent Buffer*. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Use standard within 1 hour of reconstitution.
2. Add 0.030 mL of the reconstituted standard to a tube containing 0.570 mL *Standard Diluent Buffer*. Label as 250 pg/mL Hu EGF. Mix.
3. Add 0.300 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 125, 62.5, 31.2, 15.6, 7.8, and 3.9 pg/mL Hu EGF.
4. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

B. Dilution of Human EGF Standard

Standard:	Add:	Into:
250 pg/mL	Prepare as described in Step 2.	
125 pg/mL	0.300 mL of the 250 pg/mL std.	0.300 mL of the Diluent Buffer
62.5 pg/mL	0.300 mL of the 125 pg/mL std.	0.300 mL of the Diluent Buffer
31.2 pg/mL	0.300 mL of the 62.5 pg/mL std.	0.300 mL of the Diluent Buffer
15.6 pg/mL	0.300 mL of the 31.2 pg/mL std.	0.300 mL of the Diluent Buffer
7.8 pg/mL	0.300 mL of the 15.6 pg/mL std.	0.300 mL of the Diluent Buffer
3.9 pg/mL	0.300 mL of the 7.8 pg/mL std.	0.300 mL of the Diluent Buffer
0 pg/mL	0.300 mL of the Diluent Buffer	An empty tube

Discard all remaining diluted standards after completing assay. Return the *Standard Diluent Buffer* to the refrigerator.

C. Storage and Final Dilution of Streptavidin-HRP (100X)

Please Note: The *Streptavidin-HRP (100X)* is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow *Streptavidin-HRP (100X)* to reach room temperature. Gently mix. Pipette *Streptavidin-HRP (100X)* slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 µL of this 100x concentrated solution with 1 mL of *Streptavidin-HRP Diluent* for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.

For Example:

# of 8-Well Strips	Volume of Streptavidin-HRP (100X)	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

2. Return the unused *Streptavidin-HRP (100X)* to the refrigerator.

D. Dilution of Wash Buffer

Allow the *Wash Buffer Concentrate (25X)* to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the *Wash Buffer Concentrate (25X)* with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the *Procedural Notes/Lab Quality Control* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Dilute urine samples 1:200 with *Standard Diluent Buffer* (e.g., add 20 µL of sample to 180 µL *Standard Diluent Buffer*, then add 20 µL of above dilution to 380 µL *Standard Diluent Buffer*).

   Note: Individual samples may require a greater or lesser dilution to fall within the range of the essay.

3. Add 100 µL of the *Standard Diluent Buffer* to zero wells. Well(s) reserved for chromogen blank should be left empty.

4. Add 100 µL of standards, samples or controls to the appropriate microtiter well(s). Tap gently on side of plate to mix. (See **REAGENT PREPARATION AND STORAGE**, Section B.)
5. Cover plate with *plate cover* and incubate for **2 hours at room temperature**.
6. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
7. Pipette 100 µL of biotinylated *Hu EGF Biotin Conjugate* solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
8. Cover plate with *plate cover* and incubate for **1 hour at room temperature**.
9. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
10. Add 100 µL Streptavidin-HRP Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in **REAGENT PREPARATION AND STORAGE**, Section C.)
11. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature**.
12. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
13. Add 100 µL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
14. Incubate for **30 minutes at room temperature and in the dark**. ***Please Note*: Do not cover the plate with aluminum foil or**

metalized mylar. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.

15. Add 100 µL of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
16. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.
17. Plot on graph paper the absorbance of the standards against the standard concentration. (The background absorbance may be subtracted from *all* data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
18. Read the Hu EGF concentrations for unknown samples and controls from the standard curve plotted in step 17. **Multiply value(s) obtained for urine sample(s) by 200 to correct for the 1:200 dilution in step 2.** (Samples producing signals greater than that of the highest standard (250 pg/mL) should be further diluted in *Standard Diluent Buffer* and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

TYPICAL DATA

The following data were obtained for the various standards over the range of 0 to 250 pg/mL Hu EGF.

Standard Hu EGF (pg/mL)	Optical Density (450 nm)
0	0.025
	0.027
3.9	0.074
	0.079
7.8	0.126
	0.128
15.6	0.236
	0.241
31.2	0.480
	0.473
62.5	0.836
	0.835
125	1.578
	1.600
250	2.499
	2.549

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 250 pg/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >250 pg/mL with *Standard Diluent Buffer*; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Hu EGF in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

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PERFORMANCE CHARACTERISTICS

SENSITIVITY

The minimum detectable dose of EGF is <1 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

PRECISION

1. Intra-Assay Precision

Samples of known EGF concentration were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	14.4	52.8	148
SD	0.72	2.18	5.90
%CV	5.0	4.1	4.0

SD = Standard Deviation
CV = Coefficient of Variation

2. Inter-Assay Precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	14.7	51.7	147
SD	0.78	3.03	6.70
%CV	5.3	5.9	4.6

SD = Standard Deviation
CV = Coefficient of Variation

LINEARITY OF DILUTION

Human serum and tissue culture medium containing 10% fetal bovine serum were spiked with Hu EGF and serially diluted in *Standard Diluent Buffer* over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

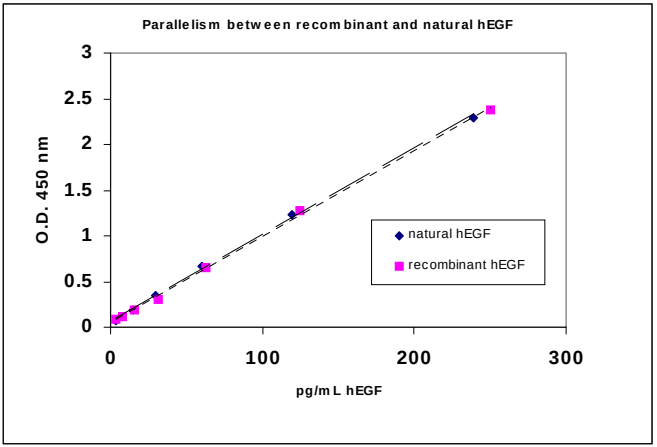
Dilution	Serum			Cell Culture		
	Measured (pg/mL)	Expected (pg/mL)	% Expected	Measured (pg/mL)	Expected (pg/mL)	% Expected
neat	197	-	-	192	-	-
1/2	91	98	93	96	96	100
1/4	51	49	104	47	48	98
1/8	27	25	108	25	24	104
1/16	13	12	108	12	12	100
1/32	6	6	100	6	6	100

RECOVERY

The recovery of Hu EGF added to human serum averaged 95%. The recovery of Hu EGF added to citrate and heparin plasma averaged 98% and 101%, respectively, while the recovery of Hu EGF added to EDTA plasma was significantly lower and is not recommended. The recovery of Hu EGF added to tissue culture medium containing 1% fetal bovine serum averaged 103%, while the recovery of Hu EGF added to tissue culture medium containing 10% fetal bovine serum averaged 106%. The recovery of Hu EGF added to urine averaged 96%.

PARALLELISM

Natural Hu EGF was serially diluted in *Standard Diluent Buffer*. The optical density of each dilution was plotted against the standard curve. Parallelism between the natural and recombinant protein was demonstrated by the figure below and indicated that the standard accurately reflects natural Hu EGF content in samples.



SPECIFICITY

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Invitrogen human EGF kit. The following substances were tested and found to have no cross-reactivity: human IL-1β, IL-2, IL-4, IL-6, IL-10, FGF acidic, FGF basic, G-CSF, GM-CSF, IFN-γ, KGF, PDGF-AB, SCF, TGF-α, TGF-β1, TNF-α, TNF-β, VEGF-121, VEGF-165; mouse IL-1β, IL-6, G-CSF, GM-CSF, IFN-γ, TNF-α; rat IL-1α, IL-6, GM-CSF, IFN-γ, TNF-α.

EXPECTED VALUES

Fifteen sera and fifteen plasma (citrate) samples from apparently normal individuals were evaluated in this assay. The values for sera ranged from 2.1 to 76 pg/mL (mean 21 pg/mL). The values for plasma ranged from 0 to 22 pg/mL (mean 9.5 pg/mL).

A limited number of commercially available pooled serum samples measured 627 to 1504 pg/mL (mean 877 pg/mL).

A limited number of urine samples from apparently normal individuals ranged from 8 to 19 ng/mL (mean 13.5 ng/mL).

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Explanation of symbols

Symbol	Description	Symbol	Description
	Catalogue Number		Batch code
	Research Use Only		*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer		European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

Human EGF Assay Summary