Human EGF ELISA Kit

Catalog Number KHG0061 (96 tests), KHG0062 (2 × 96 tests)

Pub. No. MAN0004013 **Rev.** A.0 (31)

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Human EGF ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human EGF in human serum, plasma, buffered solution, cell culture medium, or urine. The assay will recognize both natural and recombinant human EGF.

EGF is a member of a family of EGF-related growth factors including TGF-α, 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 160–70 kDa glycoprotein.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KHG0061 (96 tests)
Hu EGF Standard, lyophilized; contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide	25 mL
Antibody Coated Plate, 96-well strip-well plate	1 plate
Hu EGF Biotin Conjugate; contains 0.1% sodium azide	11 mL
Streptavidin-HRP (100X)	0.15 mL
Streptavidin-HRP Diluent; contains 3.3 mM thymol	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	3

Materials required but not supplied

- Distilled or deionized water
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Pre-dilute samples

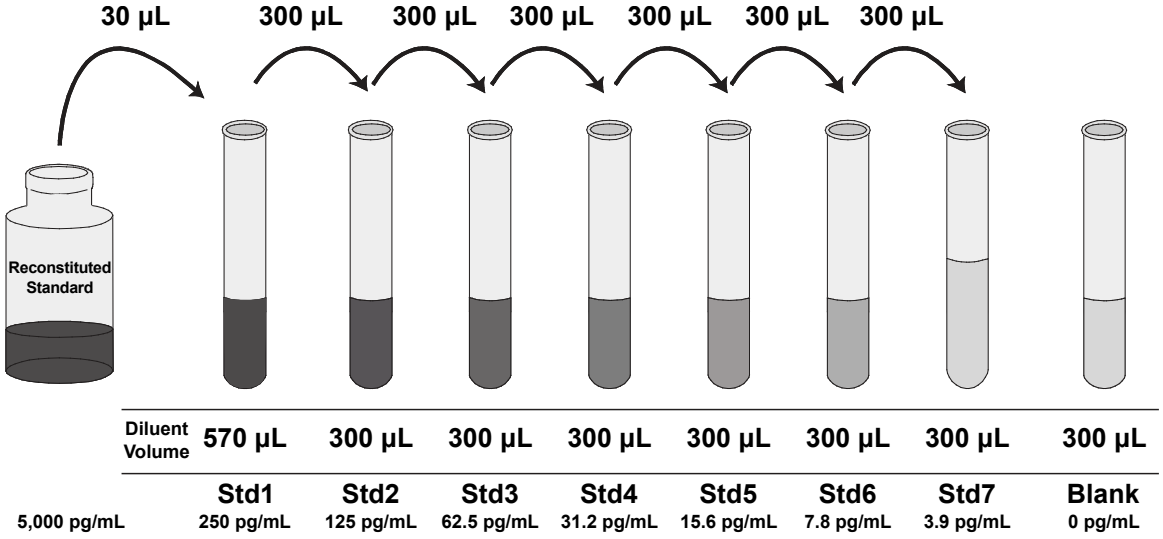
Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- Dilute samples >250 pg/mL with Standard Diluent Buffer.
- Dilute urine samples 1:200 with Standard Diluent Buffer

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Hu EGF Standard to 5,000 pg/mL with Standard Dilution Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 5,000 pg/mL human EGF. **Use the standard within 1 hour of reconstitution.**
2. Add 30 µL Reconstituted Standard to one tube containing 570 µL Standard Diluent Buffer and mix. Label as 250 pg/mL human EGF.
3. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 125, 62.5, 31.2, 15.6, 7.8, 3.9, and 0 pg/mL human EGF.
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

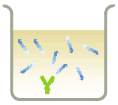
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen



a. Add 100 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Biotin Conjugate

a. Add 100 µL Hu EGF Biotin Conjugate solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 1 hour at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 Add Streptavidin-HRP

a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

4 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

5 Add Stop Solution

Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

Standard Human EGF (pg/mL)	Optical Density (450 nm)
250	2.52
125	1.59
62.5	0.84
31.2	0.48
15.6	0.24
7.8	0.13
3.9	0.08
0	0.03

Inter-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	14.7	51.7	147
Standard Deviation	0.78	3.03	6.70
% Coefficient of Variation	5.3	5.9	4.6

Intra-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	14.4	52.8	148
Standard Deviation	0.72	2.18	5.90
% Coefficient of Variation	5.0	4.1	4.0

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 1,504 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).

Sample	Range (pg/mL)	Average (pg/mL)
Serum (n=15)	2.1–76	21
Plasma (n=15)	0–22	9.5
Serum (n=Limited)	627–1,504	877
Urine Samples (n=Limited)	8,000–19,000	13,500

Linearity of dilution

Human serum and cell culture medium containing 10% fetal bovine serum were spiked with human 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		Measured (pg/mL)	Expected	
		(pg/mL)	%		(pg/mL)	%
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

Sample	Correlation Coefficient
Serum	0.99

Sensitivity

The analytical sensitivity of human 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.

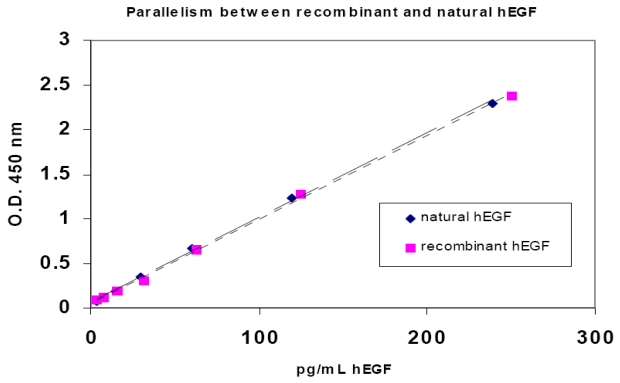
Recovery

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

Sample	Average % Recovery
Serum	95.0
Citrate and Heparin plasma	99.5
Cell culture medium (1% fetal bovine serum)	103.0
Cell culture medium (10% fetal bovine serum)	106.0
Urine	96.0

Parallelism

Natural human EGF was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the standard curve. The standard accurately reflects the full human EGF content in samples.



Specificity

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Human EGF ELISA 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.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.