Basic EGF Human ELISA Kit

Enzyme-linked immunosorbent assay for quantitative detection of human EGF

Catalog Number ECH011

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Basic EGF Human ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human EGF. This assay is designed to detect and quantify the level of human EGF in serum, plasma (citrate and heparin), buffered solution, cell culture medium, or urine.

Epidermal Growth Factor (EGF) is a growth factor that stimulates cell growth, proliferation, and differentiation by binding to its receptor, EGFR. EGF is a small protein with a molecular weight of approximately 6 kDa and is primarily produced by the submandibular glands and Brunner's glands. EGF plays a critical role in wound healing and tissue regeneration by promoting the proliferation of epithelial and fibroblast cells. Dysregulation of EGF signaling is implicated in cancer development and progression, making it a significant focus of cancer research. Therapeutic applications of EGF include its use in enhancing wound healing and tissue repair.

Contents and storage

- Store kit reagents at 2–8°C.
- Immediately after use, return remaining reagents to cold storage (2–8°C).
- See the expiration date on the package.
- The kit components' expiry is guaranteed only if they are stored properly and not contaminated during repeated use.
- Do not mix components from other lots.

Components	Amount
Coated Microwell Strips	1 pouch (12 strips with 8 wells each)
Biotin-Conjugate (1X)	11 mL
Streptavidin-HRP (100X)	150 µL
Human EGF Standard, lyophilized (0.1% sodium azide); (5000 pg/mL upon reconstitution)	2 vials
Standard Diluent Buffer	25 mL
Streptavidin-HRP Diluent; contains 3.3 mM thymol	25 mL
Wash Buffer 25X	100 mL
Substrate Solution (Tetramethyl-benzidine)	25 mL
Stop Solution (1M Phosphoric acid)	25 mL
Adhesive Film	3

Required materials not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at 450 nm (620 nm as optional reference wavelength)
- Beakers, flasks, and cylinders for preparation of reagents
- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Statistical calculator with program to perform regression analysis
- Microplate shaker

Before you begin

- Equilibrate the buffer concentrates to room temperature (18–25°C), then dilute before use.
- If crystals have formed in the buffer concentrates, warm gently to dissolve the crystals.

Prepare Wash Buffer (1X)

1. Transfer 16 mL of the Wash Buffer Concentrate (25X) to a clean 500-mL graduated cylinder, then add 384 mL of glass-distilled or deionized water. Mix gently to avoid foaming.
2. Transfer to a clean wash bottle, then label as 1X Wash Buffer.
3. Store Wash Buffer (1X) at 2–8°C for up to 14 days.

Prepare 1X Streptavidin-HRP

Make a 1:100 dilution of the concentrated Streptavidin-HRP Conjugate in a clean plastic tube.

IMPORTANT! Prepare 1X Streptavidin-HRP within 15 minutes of usage.

Dilute 0.12 mL of concentrated Streptavidin-HRP conjugate with 11.88 mL of Assay Buffer, then mix thoroughly.

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

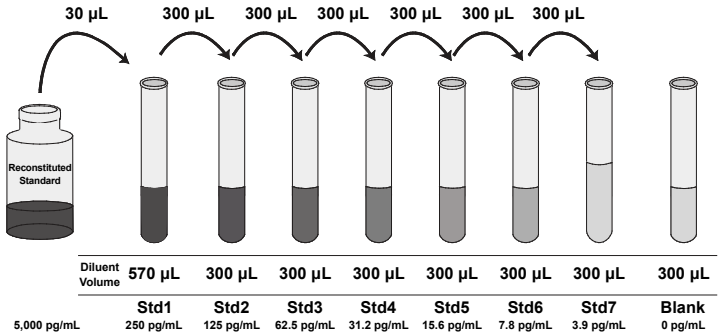
Dilute standards

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.

   IMPORTANT! Use the standard within 1 hour of reconstitution.

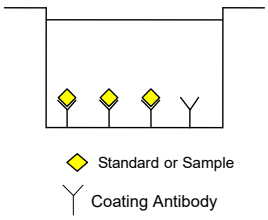
2. Add 30 µL Reconstituted Standard to one tube containing 570 µL Standard Diluent Buffer and mix. Label as 250 pg/mL human EGF.
3. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 125, 62.5, 31.2, 15.6, 7.8, 3.9, and 0 pg/mL human EGF.
4. Make serial dilutions of the standard 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.



Perform ELISA protocol

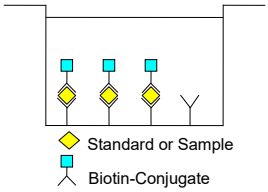
- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.

1 Bind antigen



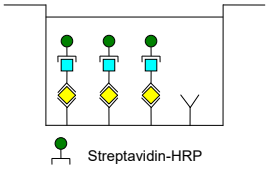
1. Add 100 µL of standards or samples (see “Pre-dilute samples” on page 2) to the appropriate wells. Leave the wells for Chromogen blanks empty.
2. Cover the plate with an adhesive film and incubate for 2 hours at room temperature.
3. Remove adhesive film and empty wells. Wash the microwell strips 4 times with approximately 400 µL of 1X Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10–15 seconds before aspiration. Do not scratch the surface of the microwells.

2 Add 1X Biotin Conjugate



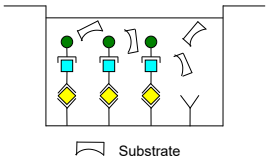
1. Add 100 µL of 1X Biotin-Conjugate solution into each well except the Chromogen blanks.
2. Cover the plate with an adhesive film and incubate for 1 hour at room temperature.
3. Prepare 1X Streptavidin-HRP as mentioned in “Prepare 1X Streptavidin-HRP” on page 2.
4. Remove adhesive film and empty wells. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer. Allow the Wash Buffer to sit in the wells for 10–15 seconds for each wash before aspiration.

3 Add 1X Streptavidin-HRP Conjugate solution



1. Add 100 µL of 1X Streptavidin-HRP Conjugate solution into each well except the Chromogen blanks.
2. Cover the plate with an adhesive film and incubate for 30 minutes at room temperature.
3. Remove adhesive film and empty wells. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer. Allow the Wash Buffer to sit in the wells for 10–15 seconds for each wash before aspiration.

4 Add TMB Substrate Solution (Stabilized Chromogen)

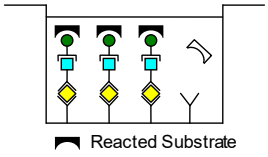


1. Add 100 µL TMB Substrate Solution to all wells.
2. Incubate the microwell strips at room temperature for about 30 minutes. Avoid direct exposure to intense light.

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

   Note: The color development on the plate should be monitored and the substrate reaction stopped (see next step) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

5 Add Stop Solution



It is recommended to add the stop solution when the highest standard develops a dark blue color.

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.

IMPORTANT! It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme.

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 and unknown samples prior to plotting.
3. Read the concentrations for unknown samples 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

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.

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.

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

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    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN1000729 A (30)

Revision	Date	Description
A (30)	9 December 2024	New document for Basic EGF Human ELISA Kit.