

Human EGF ELISA Reagent Kit

ESSHEGF

2403.1

Number	Description
ESSHEGF	<p>Human EGF ELISA Reagent Kit, pre-titered coating and detection antibodies, recommended buffers and specific assay protocol optimized for the quantitative measurement of human EGF in cell culture supernatants</p> <p>Kit provides sufficient reagents for approximately five 96-well plates, provided the Human EGF ELISA Reagent Kit Protocol is followed.</p>

Kit Contents	Size	Assay Dilution
Anti-human EGF Coating Antibody	0.625mL	1:100
Lyophilized Recombinant Human EGF Standard	5 vials	See vial label
Anti-human EGF Detection Antibody	0.625mL	1:100
Streptavidin-HRP	0.25mL	1:400
Substrate Solution	55mL	Ready-to-use
Stop Solution, 0.16M Sulfuric Acid	55mL	Ready-to-use

For research use only. Not for use in diagnostic procedures.

Storage: Immediately upon receipt, aliquot and freeze the coating and detecting antibodies at $\leq -20^{\circ}\text{C}$ in a manual defrost freezer (125 μL /tube). Avoid repeated freeze-thaw cycles. Store all other components at 2-8 $^{\circ}\text{C}$. Kit is shipped on dry ice.

Table of Contents

Introduction	1
ELISA Reagent Kit Buffers	2
Additional Materials Required.....	2
Assay Protocol.....	2
Performance Characteristics	3
General References	4

Introduction

The Thermo Scientific Human EGF ELISA Reagent Kit measures human epidermal growth factor (EGF) cytokine in serum, plasma and cell culture supernatants using the quantitative sandwich ELISA method. EGF standards and samples are captured by monoclonal anti-EGF antibody on the precoated plate and detected using a biotinylated anti-EGF antibody reactive to epitopes other than the capture antibody. The biotinylated detection antibody is then bound to streptavidin-HRP, which catalyzes the conversion of TMB to a colored derivative. Color development is linear for the assay's dynamic range and directly proportional to the amount of EGF present in the sample.

The EGF protein is proteolytically cleaved to generate the 53-amino acid EGF peptide (6-7kDa), which acts as a potent mitogenic factor. The EGF peptide is involved in the growth, proliferation and differentiation of numerous cell types. EGF defects are the cause of hypomagnesemia type 4 (HOMG4) and, in some human tumors, the EGF receptor gene has been reported to be amplified, leading to an over expression of EGF receptors on the cell surface.

Materials Required

- 8-well strip plates, clear, corner notched (Product No. 15031)
- Plate sealers for 96-well plates (Product No. 15036)
- Reagent reservoir, sterile, 50mL capacity, 40pk (Product No. 15075)

ELISA Reagent Kit Buffers

- D-PBS: 0.008M sodium phosphate, 0.002M potassium phosphate, 0.14M sodium chloride, 0.01M potassium chloride, pH 7.4, 0.2 μ m filtered (e.g., Thermo Scientific BupH Modified Dulbecco's Phosphate Buffered Saline Packs, Product No. 28374)
- Carbonate-bicarbonate Buffer: 0.2M sodium carbonate-bicarbonate buffer, pH 9.4, 0.2 μ m filtered (e.g., BupH™ Carbonate/Bicarbonate Buffer, Product No. 28382)
- Blocking Buffer: 4% BSA, 5% sucrose in D-PBS, 0.2 μ m filtered (e.g., Thermo Scientific StartingBlock (PBS) Blocking Buffer, Product No. 37538 or ELISA Blocker Blocking Buffer, Product No. N502)
- Reagent Diluent: 4% BSA in D-PBS (pH 7.4), 0.2 μ m filtered (e.g., StartingBlock™ (PBS) Blocking Buffer, Product No. 37538)
- Wash Buffer: 0.05% Tween®-20 Detergent (e.g., 0.5% Thermo Scientific Surfact-Amps 20 Detergent Solution, Product No. 28320) in D-PBS, pH 7.4 (e.g., ELISA Wash Buffer (30X), Product No. N503)
Note: Mix new solution daily.

Assay Protocol

Kit components are titrated to give optimal results using the Human EGF ELISA Reagent Kit Protocol for cell culture supernatants. Any change, including component concentration, volumes, incubation times or temperatures, buffer content or number of wash steps may significantly affect the ELISA results and require optimization to give the best results.

Note: Allow all reagents and buffers to equilibrate to room temperature (22-25°C) before use. Thaw one aliquot of coating and detecting antibody for each plate. Do not use a water bath.

A. Plate Preparation

1. Dilute the Coating Antibody 1:100 in carbonate-bicarbonate buffer by adding 110 μ L Coating Antibody to 10.89mL of carbonate-bicarbonate buffer.
2. Add 100 μ L of diluted Coating Antibody to each well. Cover plate with plate sealer and incubate overnight at room temperature.
3. Aspirate Coating Antibody solution and add 300 μ L of Blocking Buffer to each well. Cover plate with plate sealer and incubate for 1 hour at room temperature.
4. Aspirate Blocking Buffer and proceed to assay or allow to dry overnight at room temperature. When sealed with desiccant, plates can be stored at 2-8°C for 6 months.

B. Assay Procedure

1. Reconstitute standard with Reagent Diluent with volume stated on vial label. The concentration of the reconstituted standard is 5000pg/mL.
2. Dilute reconstituted standard 1:10 in Reagent Diluent to prepare top Standard (500pg/mL). Using Reagent Diluent, prepare 1:2 serial dilutions of top Standard and dilute any supernatant expected to read above the top standard. Add 100 μ L of sample or Standard to each well. Cover plate with plate sealer and incubate for 1 hour at room temperature.
3. Aspirate and wash three times with Wash Buffer using 300 μ L per well.
4. Dilute the Detection Antibody 1:100 in Reagent Diluent by adding 110 μ L of Detection Antibody to 10.89mL of Reagent Diluent.
5. Add 100 μ L of Detection Antibody to each well. Cover plate with plate sealer and incubate for 1 hour at room temperature.
6. Aspirate and wash three times with Wash Buffer, using 300 μ L per well.

7. Dilute Streptavidin-HRP 1:400 in Reagent Diluent by adding 30 μ L of Streptavidin-HRP to 12mL of Reagent Diluent.
8. Add 100 μ L of diluted Streptavidin-HRP reagent to each well. Cover plate with plate sealer and incubate for 30 minutes at room temperature.
9. Aspirate and wash three times with Wash Buffer, using 300 μ L per well.
10. Add 100 μ L of Substrate Solution to each well. Cover plate with plate sealer and incubate in the dark for 20 minutes at room temperature.
11. Stop the reaction by adding 100 μ L of Stop Solution to each well.
12. Measure the absorbance at A_{450} minus A_{550} .

C. Absorbance Measurement

Measure absorbance on an ELISA plate reader set at 450nm and 550nm. Subtract 550nm values from 450nm values to correct for optical imperfections in the microplate. If an absorbance at 550nm is not available, measure the absorbance at 450nm only.

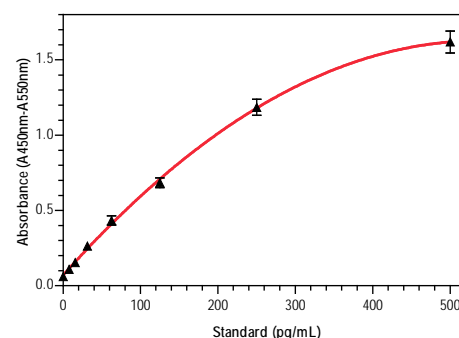
Note: When the 550nm measurement is omitted, absorbance values will be higher.

Note: Evaluate the plate within 30 minutes of stopping the reaction.

D. Calculation of Results

- The standard curve is used to determine human EGF amount in an unknown sample. Generate the standard curve by plotting the average absorbance obtained for each Standard concentration on the vertical (Y) axis vs. the corresponding human EGF concentration (pg/mL) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. Determine the human EGF amount in each sample by interpolating from the absorbance value (Y-axis) to human EGF concentration (X-axis) using the standard curve.
- If the test sample was diluted, multiply the interpolated value obtained from the standard curve by the dilution factor to calculate pg/mL of human EGF in the sample.
- Absorbance values obtained for duplicates should be within 10% of the mean value. Carefully consider duplicate values that differ from the mean by greater than 10%.

Standard Curve Example



A standard curve was generated from data obtained using the Human EGF ELISA Reagent Kit Protocol.

NOTE: This standard curve is for demonstration only. A standard curve must be run with each

Performance Characteristics

Specificity: This ELISA is specific for the measurement of natural and recombinant human EGF. No significant cross-reaction with human HB-EGF (Heparin-binding EGF), mouse EGF, rat EGF and human IL-2, IL-4, IL-6, IL-8, TNF- α , TGF- β , GM-CSF.

General References

Immunoassay: A Practical Guide. Chan and Perlstein, Eds. (1987). Academic Press: New York. p.71.

Strzepa, A., *et al.* (2011). IL-17-expressing cells as a potential therapeutic target for treatment of immunological disorders. *Pharm Reports* **63**:30-44.

Tween is a trademark of Croda International PLC.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at www.thermoscientific.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

© 2012 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.