Store at 4°C

#8628

# SignalKine™ Human EGF Chemiluminescent Sandwich ELISA Kit

☑ 1 Kit
(96 assays)

Cell Signaling TECHNOLOGY®

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New 09/12

**For Research Use Only. Not For Use In Diagnostic Procedures.**

**Entrez-Gene ID** #1950
**Swiss-Prot Acc.** #P01133

**Species Cross-Reactivity: H**

**Storage:** Following reconstitution, reagents are stable at 4°C for 1 month.

| Products Included | Volume | Solution Color |
|---|---|---|
| hEGF Rabbit mAb Coated Microwells* | 96 tests | |
| Human EGF Standard (Lyophilized) | 1 vial | |
| SignalKine™ Assay Diluent A01 | 1.5 ml | colorless |
| SignalKine™ Sample Diluent S02 | 25 ml | colorless |
| hEGF Detection Rabbit mAb (Biotinylated) (Lyophilized) 100X | 1 vial | |
| Detection Antibody Diluent | 6 ml | green |
| HRP-linked Streptavidin (Lyophilized) 100X | 1 vial | |
| HRP Diluent | 6 ml | red |
| ELISA Wash Buffer (20X) | 25 ml | colorless |
| Luminol/Enhancer Solution | 3 ml | colorless |
| Stable Peroxide Buffer | 3 ml | colorless |
| Sealing Tape | 2 sheets | |

* 12 8-well modules -Each module is designed to break apart for 8 tests.

**Description:** SignalKine™ Human EGF Chemiluminescent Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects human EGF (hEGF) in multiple matrices. Unknown samples being tested for hEGF and hEGF standards are added to low volume microwells, where the hEGF is captured by the coated hEGF Rabbit mAb. Following a washing step, a biotinylated hEGF Detection Rabbit mAb is added to detect the captured hEGF. HRP-linked Streptavidin is then used for detection of the biotinylated hEGF Detection Rabbit mAb. A chemiluminescent reagent is added for signal development. The magnitude of light emission, measured in relative light units (RLU) is proportional to the quantity of human EGF in the sample.

SignalKine™ Human EGF Chemiluminescent Sandwich ELISA Kit detects hEGF in multiple matrices that can be quantified by generating a standard curve with the recombinant hEGF protein standard provided. The hEGF standard range is from 0.6 to 2500 pg/ml. Samples containing higher levels of hEGF can be diluted to fit into the standards range.

**Matrices Tested:** This ELISA has been validated in cell culture supernatants, plasma (citrate), plasma (EDTA), plasma (heparin), serum, and urine.

**Background:** Epidermal growth factor (EGF) is a small polypeptide hormone that has mitogenic properties *in vivo* and *in vitro*, modifying the growth and/or differentiation of many cell types. EGF elicits biologic responses by binding to its cell surface receptor, a transmembrane glycoprotein containing a cytoplasmic protein tyrosine kinase domain (1,2). The binding of EGF to the EGF receptor promotes dimerization of the receptor, autophosphorylation, and activation of downstream signaling components (3). The integrated biological responses to EGF signaling are pleiotropic, including mitogenesis, apoptosis, enhanced cell motility, protein secretion, differentiation, and dedifferentiation. In addition, EGF has been implicated in organ morphogenesis, maintenance, and repair. Moreover, activation of EGF receptor signaling has been correlated with progression to invasion and metastisis in a wide variety of tumors (4-6). Research studies have identified EGF receptor and its downstream signaling molecules as potential targets for therapeutic interventions in wound repair and cancer (4-6).

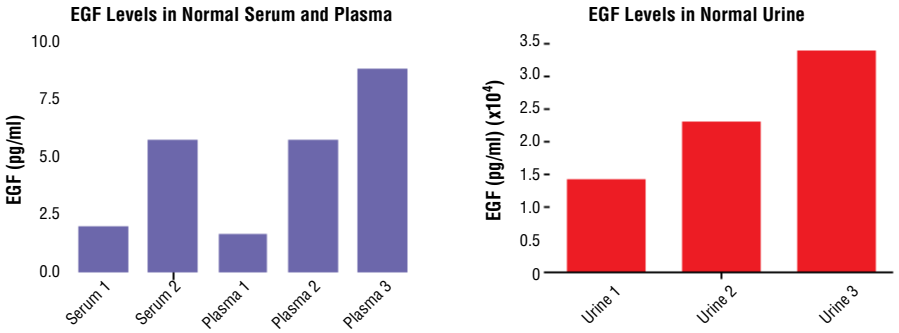



*Figure 1. hEGF levels in normal patient serum, plasma, and urine samples were quantified using SignalKine™ Human EGF Chemiluminescent Sandwich ELISA Kit.*

**Background References:**

(1) Wells, A. (1999) *Int. J. Biochem. Cell. Biol.* 31, 637-643.

(2) Boulougouris, P. and Elder, J. (2001) *Anticancer Res.* 21, 2769-2775.

(3) Schlessinger, J. (2002) *Cell* 110, 669-672.

(4) Sarries, C. et al. (2002) *Pharmacogenomics* 3, 763-780.

(5) Lorimer, I.A. (2002) *Curr. Cancer Drug Targets* 2, 91-102.

(6) Ghaneh, P. et al. (2002) *J. Hepatobiliary Pancreat. Surg.* 9, 1-11.



**Applications Key:** **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
**Species Cross-Reactivity Key:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

**Sensitivity/Lower Limit of Detection (LLD):** (< 0.54 pg/ml) Multiple assays were evaluated to calculate Sensitivity/LLD. The standard curve was run along with replicates of the zero standard. Sensitivity/LLD was determined by calculating the mean signal of the zero replicates + 2 standard deviations. This value was read off the standard curve and the concentration was determined.

The LLD range for this SignalKine™ Human EGF Chemiluminescent Sandwich ELISA Kit was 0.23 – 0.73 pg/ml. The mean LLD was 0.54 pg/ml.

**Calibration:** The hEGF standard used in this ELISA is a recombinant human EGF Asn971-Arg1023 (Accession # NM_0011963) which was produced in *E. coli* at Cell Signaling Technology (Human EGF#8916).

A hEGF, rDNA-derived standard (91/530) obtained from the WHO/NIBSC was evaluated in our assay. A conversion factor relating the CST and the WHO/NIBSC hEGF was determined. The following formula can be used to calculate results from the SignalKine™ Human EGF Chemiluminescent Sandwich ELISA Kit to the NIBSC values in International Units/ml (IU/ml):

SignalKine™ Human EGF Chemiluminescent Sandwich ELISA Kit value (pg/ml) x 0.0005 = NIBSC (91/530) value (IU/ml)

1 µg WHO/NIBSC hEGF = 2.0 µg CST hEGF.

**Specificity:** A panel of recombinant cytokines and growth factors at 10 ng/ml were tested on this ELISA. No cross-reactivity was detected with the following recombinant cytokines and growth factors:

mpro-EGF, hHB-EGF, rEGF, mEGF, hBAFF, hFGF, hFLT3LG, hG-CSF, hHGF, hIFN-γ, hIL-1α, hIL-1β, hIL-2, hIL-3, hIL-4, hIL-6, hIL-10, hIL-13, hIL-17A, hIL-17A/F, hIL-17F, hLeptin, hPDGF-AA, hPDGF-BB, hSCF, hTGF-β1, hTGF-β2, hTGF-β3, hTNF-α, mIL-2, mIL-3, mIL-4, mIL-6, mIL-10, mTNF-α.

There is a 1.25% cross reactivity with recombinant pro-hEGF in this assay.

**Spike/Recovery:** Recombinant hEGF was spiked into cell culture supernatant, plasma, and serum at three different concentrations. The percent recovery is determined by comparing hEGF concentrations, using this ELISA, with expected concentrations.

For urine samples, higher and lower hEGF containing samples were mixed together at different ratios to give high, medium and low hEGF concentrations. 100% recoveries were seen in the urine sample mixes.

| **% Recovery** | | | |
|---|---|---|---|
| Expected Concentrations | 20 pg/ml | 100 pg/ml | 1500 pg/ml |
| Cell Culture Supernatant | 95.0 | 93.4 | 107.1 |
| Plasma (Citrate) | 111.1 | 113.0 | 88.0 |
| Plasma (EDTA) | 117.2 | 104.5 | 92.5 |
| Plasma (Heparin) | 111.4 | 108.1 | 96.0 |
| Serum | 114.9 | 104.4 | 99.2 |

**Linearity of Dilution:** Recombinant hEGF spiked into various matrices and then serially diluted with SignalKine™ Sample Diluent S02 or cell culture media. Percent recoveries were then determined to demonstrate the ELISA's linearity with dilution.

| **% Recovery** | | | | | | |
|---|---|---|---|---|---|---|
| hEGF (pg/ml) | Cell Culture Supernatant | Plasma (Citrate) | Plasma (EDTA) | Plasma (Heparin) | Serum | Urine |
| 1600 | 115.2 | 102.3 | 115.4 | 119.9 | 115.0 | 109.4 |
| 400 | 106.4 | 89.7 | 92.7 | 91 | 95.2 | 98.8 |
| 100 | 98.1 | 94.3 | 85.8 | 96.9 | 90.2 | 101.1 |
| 25 | 96.7 | 95.9 | 84.7 | 94.9 | 90.9 | 100.5 |
| 6.25 | 103.4 | 87.9 | 82.7 | 91.4 | 93.3 | 96.2 |

**Precision/Reproducibility:**

*Intra-assay Precision* - Multiple replicates at three concentrations looking at the cell culture supernatant, plasma (citrate), plasma (EDTA), plasma (heparin), serum, and urine assays were run on a single plate and the values were assessed for precision/reproducibility.

*Inter-Assay Precision* - Multiple replicates at three concentrations looking at the cell culture supernatant, plasma (citrate), plasma (EDTA), plasma (heparin), serum, and urine assays were run on multiple plates over multiple days and assessed for precision/reproducibility.

| **Precision/Reproducibility** | | | | | | |
|---|---|---|---|---|---|---|
| **Cell Culture Supernatent Assay** | **Intra-Assay Precision** | | | **Inter-Assay Precision** | | |
| Expected Conc. (pg/ml) | 25 | 300 | 1500 | 25 | 300 | 1500 |
| Average (pg/ml) | 24.7 | 296.8 | 1562.9 | 24.7 | 278.8 | 1445.7 |
| Std Deviation | 1.4 | 13.8 | 145.6 | 3.4 | 20.4 | 105.4 |
| CV (%) | 5.6 | 4.6 | 9.3 | 13.7 | 7.3 | 7.3 |
| **Cell Culture Supernatant, Plasma (Citrate), Plasma (EDTA), Plasma (Heparin), Serum, Urine Assay** | **Intra-Assay Precision** | | | **Inter-Assay Precision** | | |
| Expected Conc. (pg/ml) | 25 | 300 | 1500 | 25 | 300 | 1500 |
| Average (pg/ml) | 26.8 | 284.1 | 1471.2 | 30.1 | 290.3 | 1381.1 |
| Std Deviation | 1.5 | 12.2 | 138.6 | 2.4 | 22.4 | 29.9 |
| CV (%) | 5.7 | 4.3 | 9.4 | 8.0 | 7.7 | 2.2 |

# SignalKine™ Sandwich ELISA Protocol

This ELISA was developed for the detection and quantification of cytokines, chemokines, and growth factors in cell culture media, serum, plasma, and urine. Recombinant protein standards are run alongside samples. Concentration of target in samples can be determined from the standard curve. We recommend running replicates of standards and samples.

## Reagent Preparation

**All reagents should be brought to room temperature before use.**

**Wash Buffer:** Prepare 1X wash buffer by diluting 20X wash buffer in $dH_2O$.

**SignalKine™ Assay Diluent A01:** Buffered solution supplied as a 5X solution. 10 µl of assay diluent is added to each well before addition of 40 µl of standard and samples.

**SignalKine™ Sample Diluent S02:** Diluent for diluting standards and samples when working with plasma (citrate), plasma (heparin), plasma (EDTA), serum, and urine samples.

**hEGF Detection Rabbit mAb (Biotinylated):** Supplied lyophilized. Reconstitution with 60 µl of $dH_2O$ yields a 100X Concentrated Stock solution. Incubate at room temperature for 15 min with occasional gentle mixing to fully reconstitute. Dilute 100X solution 1:100 in Detection Antibody Diluent (green solution). A 96 well plate will need 50 µl/well of 1X solution, so approximately 5 ml of reagent is needed. Dilute 50 µl of 100X stock into 5 ml of Detection Antibody Diluent or dilute as much as needed if not using full 96 well plate.

**HRP-linked Streptavidin:** Supplied lyophilized. Reconstitution with 60 µl of $dH_2O$ yields a 100X concentrated stock solution. Incubate at room temperature for 15 min with occasional gentle mixing to fully reconstitute. Dilute 100X solution 1:100 in HRP Diluent (red solution). A 96 well plate requires 50 µl/well of 1X solution, so approximately 5 ml of reagent is needed. Dilute 50 µl of 100X stock into 5 ml of HRP Diluent or dilute as much as needed if not using full 96 well plate.

**Chemiluminescent Detection Substrate:** Working Solution is prepared by mixing equal parts of **Luminol/Enhancer Solution** and **Stable Peroxide Buffer**. 50 µl of Working Solution is added to each well. A plate-based luminometer is used to measure Relative Light Units (RLU) at 425 nM within 1-10 min following addition of substrate.

Optimal signal intensity is achieved when read within 10 min. Longer periods between addition of substrate and reading plate may result in decreased signal intensity.

Working Solution is stable for 24 hr at room temperature.

## Human EGF Standard Preparation

Add 0.1 ml $dH_2O$ to reconstitute Human EGF Standard (Lyophilized) (10,000 pg) to yield a 100,000 pg/ml solution. Let sit at room temperature for 15 min with occasional gentle mixing to fully reconstitute standard protein.

For detection in plasma (citrate), plasma (heparin), plasma (EDTA), serum, and urine, samples and protein standards are diluted with **SignalKine™ Sample Diluent S02** (provided in kit). For cell culture media, samples and protein standards are diluted in **fresh cell culture media** corresponding to sample media (not supplied).

A standard range of 2500, 625, 156.3, 39, 9.8, 2.4, 0.6 and 0 pg/ml is set up. This can be achieved by diluting the protein standard, (100,000 pg/ml) 1:40, which yields 2500 pg/ml. From the 2500 pg/ml solution, serial four-fold dilutions are performed to achieve the other standard concentrations.

We recommend diluting 12.5 µl of the 100,000 pg/ml protein standard into 487.5 µl of **SignalKine™ Sample Diluent S02** or **cell culture medium** to yield the 2500 pg/ml standard. Serial four-fold dilutions can be achieved by setting up six tubes with 300 µl of the appropriate diluent and then serially transferring 100 µl from the preceding tube. Mix well before each transfer. Use the appropriate diluent as the 0 pg/ml standard.

## Sample Preparation

This assay has been validated with cell culture medium, plasma (citrate), plasma (EDTA), plasma (heparin), serum, and urine samples. After sample collection, store in single use aliquots at -20°C. Avoid multiple freeze/thawing.

Particulates in cell culture media should be removed by centrifugation. Note any contaminated, clotted, or hemolyzed samples and interpret these results judiciously.

Absorbance values falling within the hEGF standard curve range can be determined directly from the standard curve. Sample absorbance values higher than the 2500 pg/ml hEGF value should be diluted so that the absorbance values fall into standard curve range.

## Procedure

1. Once microwell strips have reached room temperature, take out as many strips as needed and insert into strip holder. Unused microwell strips should be resealed and stored at 4°C.
2. Add 10 µl of 5X SignalKine™ Assay Diluent A01 to each well.
3. Add 40 µl of hEGF Standards and samples to the wells. Cover with plate sealer and incubate for 2 hr at room temperature.
4. Wash plates 4 times with 1X Wash Buffer.

**Note:** Maximum wash volume is 170 µl/well. These are low volume microwells and have a smaller volume capacity than full size microwells.

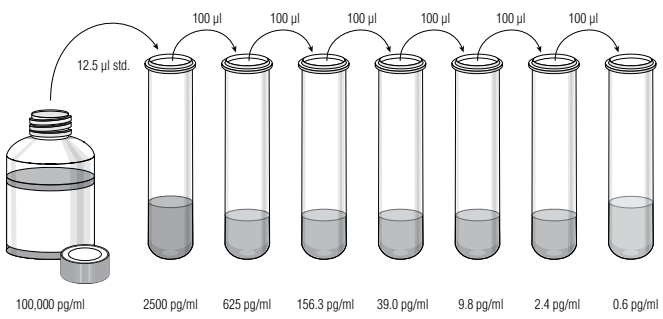
5. Add 50 µl of 1X hEGF Detection Rabbit mAb (Biotinylated) per well. Cover with plate sealer and incubate for 1 hr at room temperature.
6. Wash plates 4 times with 1X Wash Buffer.
7. Add 50 µl of 1X HRP-linked Streptavidin per well. Cover with plate sealer and incubate for 30 min at room temperature.
8. Wash plates 4 times with 1X Wash Buffer.
9. Add 50 µl of Chemiluminescent Detection Substrate Working Solution per well.
10. Use a plate-based luminometer to measure Relative Light Units (RLU) at 425 nM within 1-10 min following addition of the substrate. Optimal signal intensity is achieved when read within 10 min.

## Calculation of Results

Average replicates for standards and samples.

Using graphing/curve fitting software, plot a 4-parameter logistic curve fit. A standard curve can also be generated by plotting the standard concentration on the x-axis and the absorbance corresponding to the standard concentration on the y-axis. A best-fit curve is drawn through the points on the graph.

Sample concentrations are determined by interpolating from the standard curves. Account for sample dilution by multiplying determined concentration by dilution factor.



# SignalKine™ Sandwich ELISA Protocol

## Typical Data

Represented at right are hEGF Standard Curves, one diluted in **SignalKine™ Sample Diluent SO2** and a second diluted in cell culture media (RPMI + 10% FBS). Although these are typical of the standard curves that will be generated using this kit, a new set of standards should be run for each new experiment.