

## EGFR, Active

Human recombinant protein expressed in Sf9 cells

**Catalog # E10-112G**

Lot # D2329-8

### Product Description

Recombinant human EGFR (668-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM\\_005228](#).

### Gene Aliases

ERBB, mENA, ERBB1, HER1

### Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, and 25% glycerol.

### Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

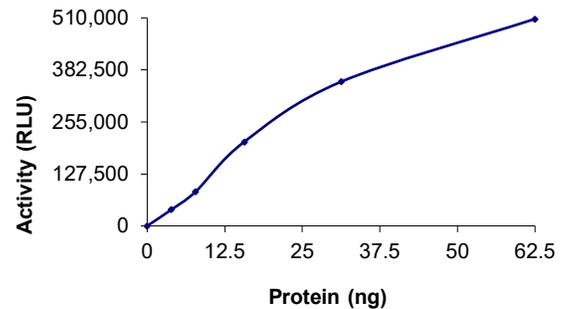
### Scientific Background

EGFR is the receptor for members of the EGF family and is a transmembrane glycoprotein that has tyrosine kinase activity. Binding of epidermal growth factor to EGFR induces receptor dimerization and tyrosine autophosphorylation and leads to cell proliferation, differentiation, motility, and cell survival. Activation of EGFR triggers mitogenic signaling in gastrointestinal mucosa, and its expression is upregulated in colon cancers and most neoplasms. Activation of EGFR triggers activation of the ERK-signaling pathway in normal gastric epithelial and colon cancer cell lines. Inactivation of EGFR with selective inhibitors significantly reduces ERK2 activation, c-fos mRNA expression and cell proliferation.

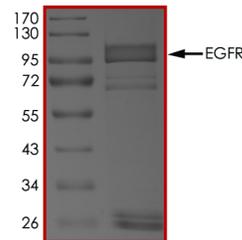
### References

1. Wang K, et al: Epidermal growth factor receptor-deficient mice have delayed primary endochondral ossification because of defective osteoclast recruitment. *J. Biol. Chem.* 279: 53848-53856, 2004.
2. Kobayashi S, et al: EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *New Eng. J. Med.* 352: 786-792, 2005.

### Specific Activity



### Purity



The purity of EGFR was determined to be **>80%** by densitometry, approx. MW **89~100kDa**.

## EGFR, Active

Catalog #	E10-112G
Specific Activity	66 nmol/min/mg
Lot #	D2329-8
Purity	>80%
Concentration	0.1 µg/µl
Stability	1yr at -70°C from date of shipment
Storage & Shipping	Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: E10-112G)

Active EGFR (0.1µg/µl) diluted with Kinase Dilution Buffer X (1x) (Catalog #: K20-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of active EGFR for optimal results).

### Kinase Assay Buffer III (5x) (Catalog #: K03-09)

Buffer components: 200mM Tris-HCl, pH 7.4, 100mM MgCl<sub>2</sub> and 0.5mg/ml BSA. Add fresh DTT prior to use to a final concentration of 250µM.

### Kinase Dilution Buffer X (1x) (Catalog #: K20-09)

Kinase Assay Buffer III (Catalog #: K03-09) with 0.1M MnCl<sub>2</sub> diluted at a 1:4 ratio (5X dilution) with cold water. Add fresh DTT to the aliquot prior to use to a final concentration of 50µM.

### ADP-Glo™ Kinase Assay Kit (Promega, Cat # V9101)

ATP solution, 10 mM  
ADP solution, 10 mM  
ADP-Glo™ Reagent  
Kinase Detection Reagent

### Substrate (Catalog #: P61-58)

Poly (4:1 Glu, Tyr) synthetic peptide substrate diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

### Cofactor: 2.5M MnCl<sub>2</sub> (Catalog #: M40-09-25)

Diluted to a working concentration of 0.1M in distilled H<sub>2</sub>O.

## Assay Protocol

The EGFR assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the EGFR reaction. The ADP-Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

- Step 1.** Thaw the Active EGFR, Kinase Assay Buffer III (5x), and Substrate on ice. Prepare a 15 µL enzyme dilution at the desired concentration, with Kinase Dilution Buffer X (1x), in a pre-chilled 96-well plate.
- Step 2.** Prepare a substrate/ATP mixture as follows (25 µM example):

Component	Amount (µL)	Component	Amount (µL)
10mM ATP Solution	1.25	Substrate at 1mg/mL	50
Kinase Assay Buffer III (5x)	46.75	0.1M MnCl <sub>2</sub>	2

- Step 3.** Transfer the following reaction components prepared in Step 2 to a 384-well opaque plate bringing the reaction volume up to 5µL:

<b>Component 1.</b>	3µl of diluted Active EGFR (Catalog # E10-112G).
<b>Component 2.</b>	2µl of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

- Step 4.** Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer X (1x).
- Step 5.** Incubate at ambient temperature for 40 minutes.
- Step 6.** After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5µl of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.
- Step 7.** Then add 10µl of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.
- Step 8.** Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader (Promega; Cat# E7061).
- Step 9.** Determine the corrected activity (RLU) by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

### Calculation of Specific Activity of ADP (RLU/pmol)

From ADP standard curve, determine RLU/pmol of ADP

### Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected RLU from reaction / [(SA of ADP in RLU/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]

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# MATERIAL SAFETY DATA SHEET

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## Article 1 - Product Identification and Use

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**Product Name: EGFR, Active**

**Catalog # E10-112G**

*This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.*

Manufacturer's Name: SignalChem Pharmaceuticals Inc.  
Street Address: 110-13120 Vanier Place  
City, Prov. Postal Code: Richmond, BC, V6V 2J2  
Fax: 604-232-4601  
EMERGENCY PHONE: 604-232-4600

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## Article 2 - Hazardous Ingredients

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NOT AVAILABLE. We are not aware of any hazards associated with this product or its ingredients, but the chemical, physical, and toxicological properties of this product have not been investigated thoroughly. Observe normal laboratory precautions.

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## Article 3 - Physical Data

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This product consists of purified protein in Tris-HCl buffer shipped on dry ice. The physical properties of this product have not been investigated thoroughly.

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## Article 4 - Fire and Explosion Hazard

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NOT APPLICABLE

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## Article 5 - Reactivity Data

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NOT APPLICABLE

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## Article 6 - Toxicologically Data

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May be harmful by inhalation, ingestion, or skin absorption. The toxicological properties of this product have not been investigated thoroughly. Exercise due caution.

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## Article 7 - Preventative Measures

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Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.

\*\*\*\*\*MULTIPLE COMPONENT SPILL OR LEAK PROCEDURES\*\*\*\*\*

- Wear protective equipment.
  - Absorb on sand or vermiculite and place in closed containers for disposal.
  - Observe all federal, state and local environmental regulations.
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## Article 8 - First Aid Measures

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- If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
  - In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. If a rash or other irritation develops, call a physician.
  - If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.
  - In case of eye contact, flush with copious amounts of water for at least 15 minutes while separating the eyelids with fingers. Call a physician.
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## Article 9 - Preparation

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Prepared By: Mya Zhang

Phone #: 1-866-954-6273

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.

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