EGFR, Active
Human recombinant protein expressed in Sf9 cells

Catalog # E10-11G
Lot # O1118-2

Product Description
Recombinant human EGFR (695-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_005228.

GeneAliases
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, 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

Specific Activity
The specific activity of EGFR was determined to be 55 nmol/min/mg as per activity assay protocol.

Purity
The purity of EGFR was determined to be >90% by densitometry, approx. MW 89kDa.

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Specific Activity

<table>
<thead>
<tr>
<th>Protein (ng)</th>
<th>Activity (cpm)</th>
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<tbody>
<tr>
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<td>0</td>
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<tr>
<td>50</td>
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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: E10-11G)
Active EGFR (0.1µg/µl) was diluted with Kinase Dilution Buffer IV (Catalog #: K24-09) 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 Dilution Buffer IV (Catalog #: K24-09)
Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with final 50ng/µl BSA solution.

Kinase Assay Buffer II (Catalog #: K02-09)
Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 20mM MgCl₂, 12.5mM MnCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

Substrate (Catalog #: P61-58)
Poly (Glu₄, Tyr₁) synthetic peptide substrate diluted in distilled H₂O to a final concentration of 1mg/ml.

[³²P]-ATP Assay Cocktail
Prepare 250µM [³²P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³²P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer II (Catalog #: K02-09). Store 1ml aliquots at −20°C.

10mM ATP Stock Solution (Catalog #: A50-09)
Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer II (Catalog #: K02-09). Store 200µl aliquots at −20°C.

Assay Protocol

Step 1. Thaw [³²P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
Step 2. Thaw the Active EGFR, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:

Component 1. 10µl of diluted Active EGFR (Catalog #E10-11G)
Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog #P61-58)
Component 3. 5µl distilled H₂O (4°C)

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
Step 5. Initiate the reaction by the addition of 5 µl [³²P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20 µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [³³P]-ATP Specific Activity (SA) (cpm/pmol)
Specific activity (SA) = cpm for 5 µl [³³P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)
Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol)*(Reaction time in min)*{(Enzyme amount in µg or mg)}*{(Reaction Volume) / (Spot Volume)}

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