

EPHA2, Active

Recombinant human protein expressed in Sf9 cells

Catalog # E14-11G

Lot # V047-1

Product Description

Recombinant human EPHA2 (561-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_004431](#).

Gene Aliases

ECK

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 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.

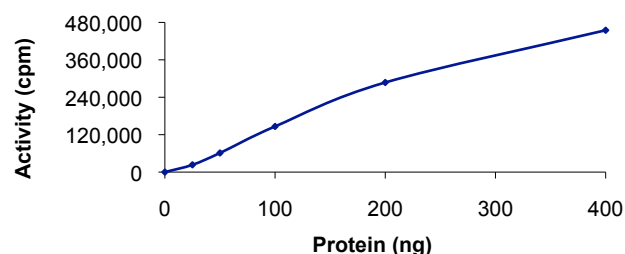
Scientific Background

EPHA2 is a member of the ephrin receptor subfamily of protein-tyrosine kinases that bind the ephrin-A ligand and have diverse cellular function. EPHA2 has been shown to be an oncoprotein of importance in a range of cancers. EPHA2 is overexpressed in several human cancer types and promotes malignancy through a mechanism involving RhoA-dependent destabilization of adherens junctions (1). EPHA2 overexpression induces a FAK-dependent increase in MMP-2 expression and invasiveness and this process that can be reversed by ligation of EPHA2 (2).

References

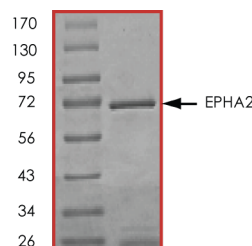
1. Fang, W B. et al: Overexpression of EPHA2 receptor destabilizes adherens junctions via a RhoA-dependent mechanism. J Cell Sci. 2008 Feb 1;121(Pt 3):358-68.
2. Duxbury, M S. et al: Ligation of EphA2 by Ephrin A1-Fc inhibits pancreatic adenocarcinoma cellular invasiveness. Biochem Biophys Res Commun. 2004 Aug 6;320(4):1096-102.

Specific Activity



The specific activity of EPHA2 was determined to be **87 nmol/min/mg** as per activity assay protocol.

Purity



The purity of EPHA2 was determined to be **>90%** by densitometry, approx. MW **72kDa**.

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Catalog Number

E14-11G

Specific Activity

87 nmol/min/mg

Specific Lot Number

V047-1

Purity

>90%

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 #: E14-11G)

Active EPHA2 (0.1µg/µl) 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 EPHA2 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.

[³³P]-ATP Assay Cocktail

Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-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.

Substrate

Poly (Glu:Tyr, 4:1) synthetic peptide substrate diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active EPHA2, 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 EPHA2 (Catalog #E14-11G)
 - Component 2.** 5µl of 1mg/ml stock solution of substrate
 - 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 [³³P]-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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