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# EPHA3, Active

Recombinant human protein expressed in Sf9 cells

**Catalog # E15-11G**
*Lot # V155-1*

## Product Description

Recombinant human EPHA3 (571-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is BC063282.

## Gene Aliases

ETK, HEK, ETK1, HEK4, TYRO4

## 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.

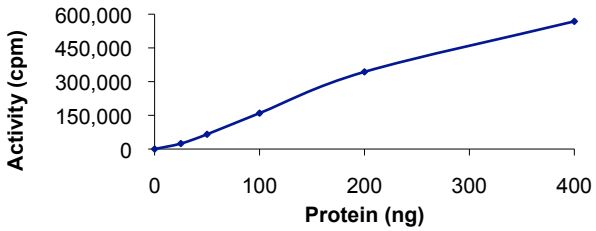
## Scientific Background

EPHA3 is a member of the ephrin receptor subfamily of protein-tyrosine kinases that bind the ephrin-A ligand and have diverse cellular function. Analysis of human colorectal, breast, lung and pancreatic cancer samples shows somatic mutations in the EPHA3 gene (1). EPHA3 gene expression can be regulated by CD28 and IGF-1 in Jurkat cells and expression of EPHA3 is associated with adherence and motility of malignant T cells (2).

## References

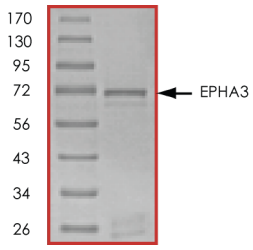
1. Wood, L D. et al: Somatic mutations of GUCY2F, EPHA3, and NTRK3 in human cancers. Hum Mutat. 2006 Oct;27(10):1060-1.
2. Smith, L M. et al: EphA3 is induced by CD28 and IGF-1 and regulates cell adhesion. Exp Cell Res. 2004 Jan 15;292(2):295-303.

## Specific Activity




The specific activity of EPHA3 was determined to be **102 nmol /min/mg** as per activity assay protocol.

## Purity




The purity of EPHA3 was determined to be **>90%** by densitometry, approx. MW **72kDa**.

# EPHA3, Active

Recombinant human protein expressed in Sf9 cells

| | |
|---|---|
| Catalog Number | E15-11G |
| Specific Activity | 102 nmol/min/mg |
| Specific Lot Number | V155-1 |
| 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. |

# Activity Assay Protocol

## Reaction Components

**Active Kinase** (Catalog #: E15-11G)

Active EPHA3 (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 EPHA3 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 $MgC1_2$, 12.5mM $MnC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**[$^{33}$P]-ATP Assay Cocktail**

Prepare 250μM [$^{33}$P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [$^{33}$P]-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 $H_2O$ to a final concentration of 1mg/ml.

## Assay Protocol

**Step 1.** Thaw [$^{33}$P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

**Step 2.** Thaw the Active EPHA3, 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 EPHA3 (Catalog #E15-11G)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate
- **Component 3.** 5μl distilled $H_2O$ (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 $H_2O$.

**Step 5.** Initiate the reaction by the addition of 5 μl [$^{33}$P]-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 $H_2O$) 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 [P$^{33}$]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [$^{33}$P]-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 $^{33}$P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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