FAK, Active
Recombinant human protein expressed in Sf9 cells

Catalog # P91-11H
Lot # X694-1

Product Description

Recombinant human FAK (393-698) was expressed by baculovirus in Sf9 insect cells using an N-terminal His tag. The gene accession number is NM_153831.

Gene Aliases
PTK2, FADK, FAK1, pp125FAK

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, 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

FAK (Focal Adhesion Kinase) is a non-receptor protein tyrosine kinase involved in signal transduction from integrin-enriched focal adhesion sites that mediate cell contact with the extracellular matrix. FAK-enhanced signals have been shown to mediate the survival of anchorage-dependent cells and are critical for efficient cell migration in response to growth factor receptor and integrin stimulation (1). Elevated expression of FAK in human tumors has been correlated with increased malignancy and invasiveness (2). Elevated FAK expression in anaplastic astrocytoma and glioblastoma tumor biopsy samples has been demonstrated.

References


Specific Activity

The specific activity of FAK was determined to be 285 nmol/min/mg as per activity assay protocol.

Purity

The purity was determined to be >75% by densitometry. Approx. MW 35kDa.

FAK, Active
Recombinant human protein expressed in Sf9 cells

Catalog Number P91-11H
Specific Activity 285 nmol/min/mg
Specific Lot Number X694-1

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. Product shipped on dry ice.

To place your order, please contact us by phone 1-604-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com

www.signalchem.com

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.
Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: P91-11H)
Active FAK (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 FAK 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 50 ng/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 (4:1 Glu, Tyr) synthetic peptide substrate diluted in distilled H2O to a final concentration of 1mg/ml.

[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.

Assay Protocol

Step 1. Thaw [33P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
Step 2. Thaw the Active FAK, 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 FAK (Catalog # P91-11H)
   - Component 2. 5µl of 1 mg/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 5µl [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 20µl 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 [P³³]-ATP Specific Activity (SA) (cpm/pmol)
Specific activity (SA) = cpm for 5µl [³³P]-ATP / pmoles of ATP (in 5µl of a 250µM 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)]

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.