FAK, Active
Recombinant human protein expressed in Sf9 cells

Catalog # P91-11H
Lot # X383-1

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, 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
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 51 nmol/min/mg as per activity assay protocol, and was equivalent to 291.5 nmol/min/mg as per radiometric assay.

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

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Catalog # P91-11H
Specific Activity 51 nmol/min/mg
Lot # X383-1
Purity >75%
Concentration 0.05 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 #: P91-11H)
Active FAK (0.05µg/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 FAK for optimal results).

**Kinase Assay Buffer III (5x)** (Catalog #: K03-09)
Buffer components: 200mM Tris-HCl, pH 7.4, 100mM MgCl2 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 12.5mM MnCl2 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-GloTM 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 H2O to a final concentration of 1mg/ml.

**Cofactor: 2.5M MnCl2** (Catalog #: M40-09-25)
Diluted to a working concentration of 0.1M in distilled H2O.

Assay Protocol

The FAK assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the FAK 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.

1. Thaw the Active FAK, 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.
2. Prepare a substrate/ATP mixture as follows (25 µM example):

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (µL)</th>
<th>Component</th>
<th>Amount (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10µM ATP Solution</td>
<td>1.25</td>
<td>Substrate at 1mg/mL</td>
<td>50</td>
</tr>
<tr>
<td>Kinase Assay Buffer III (5x)</td>
<td>46.75</td>
<td>0.1M MnCl2</td>
<td>2</td>
</tr>
</tbody>
</table>

3. Transfer the following reaction components prepared in Step 2 to a 384-well opaque plate bringing the reaction volume up to 5µL:
   - Component 1: 3µl of diluted Active FAK (Catalog # P91-11H).
   - Component 2: 2µl of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

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).
5. Incubate at ambient temperature for 40 minutes.
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.
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.
8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader (Promega; Cat# E7061).
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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