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Certificate of Analysis

FAK, active

(Recombinant enzyme expressed in Sf21 insect cells)

Catalogue # 14-720

Lot # 1610234

Product Description: N-terminal 6His-tagged, recombinant, human FAK, amino acids 411–686, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 89% by SDS-PAGE and Coomassie blue staining. MW = 35.5kDa.

Specific Activity (lot# 1610234): 106U/mg, where one unit of FAK activity is defined as 1nmol phosphate incorporated into 100µM (EEEEEEEEEEEEYYIIEEEEEEEEEEEEEEEEYYEEEEEEEEKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 10µg of enzyme in 5.2µl of 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

Storage and Stability: Stable for 6 months at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

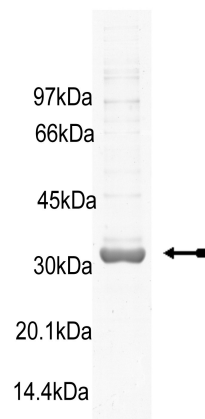
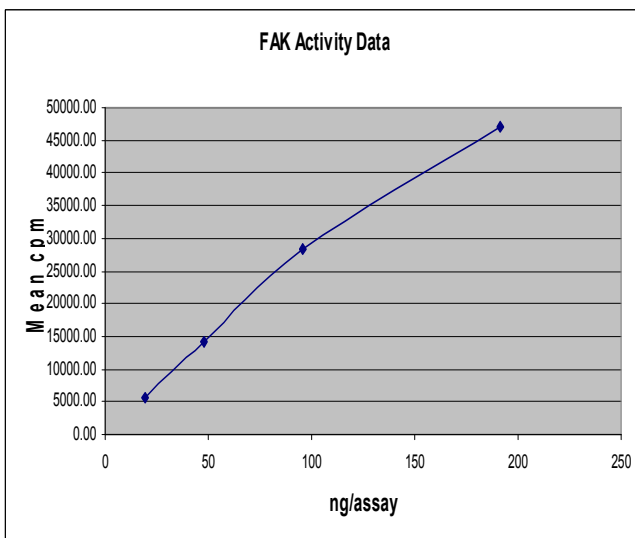
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 19.0–191.6ng of this lot of enzyme phosphorylated 100µM (EEEEEEEEEEEEYYIIEEEEEEEEEEEEEEEEYYEEEEEEEEKK) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.

MS Tryptic Fingerprint: Confirmed identity as FAK with 54% amino acid coverage of the translated native sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of FAK, active.



Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **(EEEEEEEEEEEEYYIIEEEEEEEEEEEEEEEEEEEE EEEKKK):** Use at a final assay concentration of 100 μ M. Make up a 1mM stock in 100mM Hepes pH8.0. Add 2.5 μ l of stock per assay point.
3. **FAK, active:** Dilute with 20mM MOPS/NaOH pH7.0, 150mM NaCl, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 19.0–191.6ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure(96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of (EEEEEEEEEEEEYYIIEEEEEEEEEEEEEEEEEEEE EEEKKK).
3. Add 2.5 μ l (19.0–191.6ng), FAK, active.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

FAK Sequence Information

<u>Protein</u>	Human FAK
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	S31 of the recombinant protein is equivalent to S411 of human FAK
<u>Accession number</u>	GenBank L13616

Recombinant FAK amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF STRDYEIQRE RIELGRCIGE GQFGDVHQGI
61 YMSPENPALA VAIKTCKNCT SDSVREKFLQ EALTMRQFDH PHIVKCLIGVI TENPVWIIME
121 LCTLGELRSF LQVRKYSLDL ASLILYAYQL STALAYLESK RFVHRDIAAR NVLVSSNDCV
181 KLGDFGLSRY MEDSTYYKAS KGKLPKQWMA PESINFRRFT SASDVVMFGV CMWEILMHGV
241 KPFQGVKNND VIGRIENGER LPMPPNCPPT LYSLMTKCWA YDPSRRPRFT ELKAQLSTIL
301 EEEKAQ
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Recombinant FAK nucleotide sequence:

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1 atgtcgact accatcacca tcaccatcac gattacgata toccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc tcaaccaggg attatgagat tcaaagagaa
121 agaatagaac ttggacgatg tattggagaa ggccaatttg gagatgtaca tcaaggcatt
181 tatatgagtc cagagaatcc agctttggcg gttgcaatta aaacatgtaa aaactgtact
241 tcggacagcg tgagagagaa atttcttcaa gaagccttaa caatgctgca gtttgaccat
301 cctcatattg tgaagctgat tggagtcatc acagagaatc ctgtctggat aatcatggag
361 ctgtgcacac ttggagagct gaggtcattt ttgcaagtaa ggaaatacag tttggatcta
421 gcatctttga tcctgtatgc ctatcagctt agtacagctc ttgcatatct agagagcaaa
481 agatttgtag acagggacat tgctgctcgg aatgttctgg tgcctcaaaa tgattgtgta
541 aaattaggag actttggatt atcccgatat atggaagata gtacttacta caaagcttcc
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721 aagccttttc aaggagtgaa gaacaatgat gtaatcggtc gaattgaaaa tggggaaaga
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Reviewed and approved by site quality representative.

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