

Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **(EEEEEEEEEEEEYYIIEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEKKK):** Use at a final assay concentration of 100μM. Make up a 1mM stock in 100mM Hepes pH8.0. Add 2.5μl 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 66–182ng 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 5μl of 5 x reaction buffer per assay to wells.
2. Add 2.5μl of **(EEEEEEEEEEEEYYIIEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEKKK)**.
3. Add **2.5μl (66–182ng), FAK, active**.
4. Add 5μl of dH₂O.
5. Add 10μl of diluted [γ-³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5μl of 3% phosphoric acid.
8. Transfer a 10μl 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 1μl of 30% phosphoric acid.

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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 PHIVKLIGVI TENPVWIIME
121 LCTLGELRSF LQVRKYSDDL ASLILYAYQL STALAYLESK RFVHRDIAAR NVLVSSNDCV
181 KLGDGFLSRV MEDSTYYKAS KGKLPKWKMA PESINFRRFT SASDVWMFGV CMWEILMHGV
241 KPFQGVKNND VIGRIENGER LMPNPCPPT LYSMTKCWA YDPSRRPRFT ELKAQLSTIL
301 EEEKAQ

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Recombinant FAK nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac 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 caatgcgtca gtttgaccat
301 cctcatattg tgaagctgat tggagtcatt acagagaatc ctgtctggat aatcatggag
361 ctgtgcacac ttggagagct gaggtcattt ttgcaagtaa ggaaatacag tttggatcta
421 gcatttttga tcctgtatgc ctatcagctt agtacagctc ttgcatactc agagagcaaa
481 agattttgtac acagggacat tgctgctcgg aatgttctgg tgtcctcaaa tgatttgtga
541 aaattaggag actttggatt atcccgatat atggaagata gtacttacta caaagcttcc
601 aaaggaaaat tgcctattaa atggatggct ccagagtcaa tcaattttcg acgttttacc
661 tcagctagtg acgtatggat gtttgggtgt tgtatgtggg agatactgat gcatggtgtg
721 aagccttttc aaggagtga gaacaatgat gtaatcggtc gaattgaaaa tggggaaaaga
781 ttaccaatgc ctccaaattg tcctcttacc ctctacagcc ttatgacgaa atgctgggcc
841 tatgacccca gcaggcggcc caggtttact gaacttaaag ctcagctcag cacaatcctg
901 gaggaagaga aggctcagta a

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