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Standard Operating Procedure

Preparation of active PRAK

Enzyme description:- Active PRAK

Source:- Recombinant

Expression system:- Baculovirus expression vector system (BEVS)/Insect cells

Tag:- His(6)

Purification method:- Ni^{2+}-NTA agarose

Expression level:- 2-3 mg/L

Molecular mass:- 54 kDa by SDS-PAGE

Purity:- >85%

Contaminants:- The preparation contains several minor degradation products.

Activation protocol:-

PRAK (0.22 mg/ml - 4 μM) is activated in 50mM Tris/HCl pH 7.5, 0.1mM EGTA, 0.1 % β-mercaptoethanol, 0.1 mM sodium vanadate, 10 mM magnesium acetate, 0.1 mM ATP with 2 U/ml active GST-SAPK2a/p38 at 30°C for 45 min. Following activation, the PRAK is separated from the GST-SAPK2a/p38 by Ni-NTA chromatography (PRAK binds to the resin by virtue of its His-tag). The re-purified active PRAK is then eluted from the column in enzyme storage buffer and fractions are pooled and snap frozen in liquid nitrogen prior to storage at –70°C.

Enzyme storage buffer:-
50 mM Tris/HCl pH 7.5, 270 mM sucrose, 150 mM imidazole, 150 mM NaCl, 0.1 mM EGTA, 0.1 % β-mercaptoethanol, 0.02% Brij-35, 0.2 mM PMSF,1 mM Benzamidine.

Storage temperature:- Aliquot, snap freeze and store at -70°C.

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CLONE DATA SHEET – human PRAK (MAPKAP-K5)

Protein	Human PRAK (MAPKAP-K5)
Accession number	AF032437
Tags	His(6)
Baculovirus-expressed protein	MHHHHHH**MSEESDMDKAIKETSILEEYSINWTQKLGAG ISGPVRVCVKKSTQERFALKILLDRPKARNEVRLHMMC ATHPNIVQIIEVFANSVQFPHESSPRARLLIVMEMMEGG ELFHRISQHRHFTEKQASQVTKQIALALRHCHLLNIAHR DLKPENLLFKDNSLDAPVKLCDFGFAKIDQGDLMTPQF TPYYVAPQVLEAQRRHQKEKSGIIPTSPTPYTYNKSCDL WSLGVIIYVMLCGYPPFYSKHHSRTIPKDMRRKIMTGSF EFPEEEWSQISEMAKDVVRKLLKVKPEERLTIEGVLDHP WLNSTEALDNVLPSAQLMMDKAVVAGIQQAHAEQLA NMRIQDLKVSLKPLHSVNNPILRKRKLLGTKPKDSVYIH DHENGAEDSNVALEKLRDVIAQCILPQAGENEDEKLNE VMQEAWKYNRECKLLRDTLQSFSWNGRGFTDKVDRLK LAEIVKQVIEEQTTSHESQ**
Native sequence	Met8 of the fusion-protein is Met1 of PRAK. This clone harbours a R298E substitution. Inspection of the murine PRAK sequence (known as MAPKAP-K5) indicates that this residue is also a Glu, which implies that the original PRAK sequence submitted to the database has a mistake at this position.
Protease cleavage site	None
Cloning sites	Nde1/Xhol sites of modified pFastBAC 1. Xhol site immediately follows stop codon

ORF in baculovirus

ATGCACCATCACCATCACCATATGTCGGAGGAGAGCGACATGGACAAAG
CCATCAAGGAAACTTCCATTTTAGAAGAATACAGTATCAATTGGACTCA
GAAGCTGGGAGCTGGAATTAGTGGTCCAGTTAGAGTCTGTGTAAAGAAA
TCTACTCAAGAACGGTTTGCGCTGAAAATTCTTCTTGATCGTCCAAAAG
CTAGAAATGAGGTACGTCTGCACATGATGTGTGCCACACACCCAAACAT
AGTTCAGATTATTGAAGTGTTTGCTAACAGTGTCCAGTTTCCCCATGAG
TCCAGCCCTAGGGCCCGACTCTTAATTGTAATGGAGATGATGGAAGGGG
GAGAGCTATTTCACAGAATCAGCCAGCACCGGCACTTTACAGAGAAGCA
AGCCAGCCAAGTAACAAAGCAGATAGCTTTGGCTCTGCGGCACTGTCAC
TTGTTAAACATTGCGCACAGAGACCTCAAGCCTGAAAATCTGCTTTTTA
AGGATAACTCTTTGGATGCCCCAGTGAAGTTGTGTGACTTTGGATTTGC
CAAGATTGACCAAGGTGACTTGATGACACCCCAGTTCACCCCTTATTAT
GTAGCACCCCAGGTACTGGAGGCGCAAAGAAGGCATCAGAAGGAGAAAT
CTGGCATCATACCTACCTCACCGACGCCCTACACTTACAACAAGAGCTG
TGACTTGTGGTCCCTAGGGGTGATTATCTATGTGATGCTGTGCGGATAC
CCTCCTTTTTACTCCAAACACCACAGCCGGACTATCCCAAAGGATATGC
GAAGAAAGATCATGACAGGCAGTTTTGAGTTCCCAGAGGAAGAGTGGAG
TCAGATCTCAGAGATGGCCAAAGATGTTGTGAGGAAGCTCCTGAAGGTC
AAACCGGAGGAGAGACTCACCATCGAGGGAGTGCTGGACCACCCCTGGC
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GATGGACAAGGCAGTGGTTGCAGGAATCCAGCAGGCTCACGCGGAACAG
TTGGCCAACATGAGAATCCAGGATCTGAAAGTCAGCCTCAAACCCCTGC
ACTCAGTGAACAACCCCATTCTGCGGAAGAGGAAGTTACTTGGCACCAA
GCCAAAGGACAGTGTCTATATCCACGACCATGAGAATGGAGCCGAGGAT
TCCAATGTTGCCTTGGAAAAACTCCGAGATGTGATTGCTCAGTGTATTC
TCCCCCAGGCTGGAGAGAATGAAGATGAGAAACTGAATGAAGTAATGCA
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CAGAGCTTCAGCTGGAATGGTCGTGGATTCACAGATAAAGTAGATCGAC
TAAAACTGGCAGAAATTGTGAAGCAGGTGATAGAAGAGCAAACCACGTC
CCACGAATCCCAATAA