Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active PRAK [1 - 471]

Enzyme description:- PRAK [1 - 471]

Clone number:- DU 1853

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

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

Expression level:- 2-3 mg/L

Calculated molecular mass:- 54, 954 daltons

Purity:- >85 %

Activation protocol:-

PRAK (4 µM) is activated in 50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 0.1 mM sodium vanadate, 10 mM magnesium acetate, 0.1 mM ATP with 2 U/ml GST-SAPK2a [DU 979] at 30 °C for 45 min. Following activation, PRAK is re-purified by Ni^{2+}-NTA agarose chromatography.

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- -70 °C

Assay:- Standard filter binding assay

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1% 2−mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-
PRAKtide (KKLRRTLSVA) Final concentration: 30 µM

Specific activity range:- 25 – 50 U/mg

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Clone Data Sheet

PRAK [1 - 471]

Protein	PRAK [1 - 471]
Clone number	DU 1853
Species	Human
Accession number	AF032437
Tags	N-terminal His(6)
Baculovirus expressed protein	MHHHHHH**MSEESDMDKAIKETSILEEYSINWTQKLGAGISGPVRVC VKKSTQERFALKILLDRPKARNEVRLHMMCATHPNIVQIIEVFANS VQFPHESSPRARLLIVMEMMEGGELFHRISQHRHFTEKQASQVTKQ IALALRHCHLLNIAHRDLKPENLLFKDNSLDAPVKLCDFGFAKIDQ GDLMTPQFTPYYVAPQVLEAQRRHQKEKSGIIPTSPTPYTYNKSCD LWSLGVIIYVMLCGYPPFYSKHHSRTIPKDMRRKIMTGSFEFPEEE WSQISEMAKDVVRKLLKVKPEERLTIEGVLDHPWLNSTEALDNVLP SAQLMMDKAVVAGIQQAHAEQLANMRIQDLKVSLKPLHSVNNPILR KRKLLGTKPKDSVYIHDHENGAEDSNVALEKLRDVIAQCILPQAGE NEDEKLNEVMQEAWKYNRECKLLRDTLQSFSWNGRGFTDKVDRLKL AEIVKQVIEEQTTSHESQ**
Native sequence	Amino acids M1 – Q471 (end) of human PRAK. Residue M8 of the fusion protein is equilivalent to M1 of the native enzyme. The His(6) tag is located at residues 2 – 7.
Protease cleavage	None
Cloning sites	*Nde*1 and *Xho*l sites of modified pFastBAC 1

Nucleotide sequence of insert

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