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Standard Operating Procedure

Preparation of active S6K1/ p70S6Kinase

Enzyme description:- **Active Human P70 S6 kinase (1-421) T412E**

Source:- Recombinant

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

Tag:- His(6)

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

Expression level:- 3-5 mg/L

Molecular mass:- ~50kDa by Novex gel

Purity:- >85%

Contaminants:- No major contaminating proteins as judged by SDS-PAGE.

Activation protocol:-

S6K1/ P70S6 Kinase (1-421)T412E (0.11 mg/ml – 2 μM) is activated by incubation with 1μg/ml PDK1 in 50mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % β-mercaptoethanol, 10 mM magnesium acetate, 0.1 mM ATP for 30 min at 30°C. Following activation, the preparation is made 400 mM NaCl and incubated with Heparin Sepharose. The active S6K1/ P70S6K is collected in the flow through (PDK1 binds Heparin Sepharose at 400 mM NaCl) and dialysed into enzyme storage buffer.

Enzyme storage buffer:-

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

Storage temperature:- Snap freeze and store at -80°C

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CLONE DATA SHEET – human S6K1/ p70S6 Kinase

Protein	Human P70 S6 kinase (1-421) T412E
Accession number	NP_003152
Tags	His(6)
Baculovirus-expressed protein	MHHHHHHMRRRRRRDGFYPAPDFRHREAEDMAGVFDI DLDQPEDAGSEDELEEGGQLNESMDHGGVGPYELGMEH CEKFEISETSVNRGPEKIRPECFELLRVLGKGGYGKVFQVR KVTGANTGKIFAMKVLKKAMIVRNAKDTAHTKAERNILE EVKHPFIVDLIYAFQTGGKLYLILEYLSGGELFMQLEREGIF MEDTACFYLAEISMALGHLHQKGIIYRDLKPENIMLNHQ GHVKLTDFGLCKESIHDGTVTHTFCGTIEYMAPEILMRSG HNRAVDWWSLGALMYDMLTGAPPFTGENRKKTIDKILK CKLNLPPYLTQEARDLLKKLLKRNAASRLGAGPGDAGEV QAHPFFRHINWEELLARKVEPPFKPLLQSEEDVSQFDSKFT RQTPVDSPDDSTL SESANQVFLGFEYVAPSVLES
Native sequence	Residue 8 of the His_6-tagged protein is equivalent to Met1 of S6K1/P70 S6 kinase. This sequence differs from the database entry with a D18H substitution.
Protease cleavage site	None
Cloning sites	BamHI/Not1 sites of pFastBAC1

ORF in baculovirus

ATGCACCATCACCATCACCATATGAGGCGACGAAGGAGGCGGGAC
GGCTTTTACCCAGCGCCTGACTTCCGACACAGGGAAGCTGAGGAC
ATGGCAGGAGTGTTTGACATAGACCTGGACCAGCCAGAGGATGCA
GGCTCTGAGGATGAGCTGGAGGAGGGGGGTCAGTTAAATGAAAGC
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AAAGGGGGCTATGGAAAGGTTTTTCAAGTACGAAAAGTAACAGGA
GCAAATACTGGGAAGATATTTGCCATGAAGGTGCTTAAAAAGGCA
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GTGGCTCCATCTGTACTTGAAAGTTGA