Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active S6 Kinase 1 T412E [1 – 421]

Enzyme description:- S6 kinase 1 T412E [1 - 421]

Clone number:- DU 784

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

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

Expression level:- 3-5 mg/L

Calculated molecular mass:- 48, 554 Daltons

Purity:- >85 %

Activation protocol:-
S6K1 (2 µM) is activated by incubation with 1 µg/ml GST-PDK1 [DU 954] in 50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc, 0.1 mM ATP for 30 min at 30 °C. Following activation, S6K1 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, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF.

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:-
CROSStide (GRPRTSSFAEG) Final concentration: 30 µM

Specific activity range:- 70 – 140 U/mg

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Clone Data Sheet

S6 Kinase 1 T412E [1 – 421]

Protein S6 Kinase 1 T412E [1 - 421]

Clone number DU 784

Species Human

Accession number NM_003161

Tags N-terminal His(6)

Baculovirus expressed protein

MHHHHHH**MRRRRRRDGFYPAPDFRHREAEDMAGVFDIDLDQPEDAGS
EDELEEGGQLNESMDHGGVGPYELGMEHCEKFEISETSVNRGPEKIR
PECFELLRVLGKGGYGKVFQVRKVTGANTGKIFAMKVLKKAMIVRNA
KDTAHTKAERNILEEVKHPFIVDLIYAFQTGGKLYLILEYLSGGELF
MQLEREGIFMEDTACFYLAEISMALGHLHQKGIIYRDLKPENIMLNH
QGHVKLTDFGLCKESIHDGTVTHTFCGTIEYMAPEILMRSGHNRAVD
WWSLGALMYDMLTGAPPFTGENRKKTIDKILKCKLNLPPYLTQEARD
LLKKLLKRNAASRLGAGPGDAGEVQAHPFFRHINWEELLARKVEPPF
KPLLQSEEDVSQFDSKFTRQTPVDSPDDSTLSESANQVFLGFEYVAP
SVLES**

Native sequence

Amino acids M1 – S421 of human S6 kinase 1.
[Full length protein ends at residue L525]
Residue M8 of the fusion protein is equivalent to M1 of the native enzyme. The enzyme has a T412E mutation to mimic activation of the PDK2 site. Residue T412 is equilivalent to E419 of the fusion protein. The His(6) tag is located at residues 2 – 7.

The following amino acid substitution is present:
D – H, where D18 of the native sequence is H25 of the fusion protein.

Protease cleavage None

Cloning sites *Bam*HI and *Not*1 sites of pFastBAC1

Nucleotide sequence of insert

ATGCACCATCACCATCACCATATGAGGCGACGAAGGAGGCGGGACGG
CTTTTACCCAGCGCCTGACTTCCGACACAGGGAAGCTGAGGACATGG
CAGGAGTGTTTGACATAGACCTGGACCAGCCAGAGGATGCAGGCTCT
GAGGATGAGCTGGAGGAGGGGGGTCAGTTAAATGAAAGCATGGACCA
TGGGGGAGTTGGACCATATGAACTTGGCATGGAACATTGTGAGAAAT
TTGAAATCTCAGAAACTAGTGTGAACAGAGGGCCAGAAAAAATCAGA
CCAGAATGTTTTGAGCTACTTCGGGTACTTGGTAAAGGGGGCTATGG
AAAGGTTTTTCAAGTACGAAAAGTAACAGGAGCAAATACTGGGAAGA
TATTTGCCATGAAGGTGCTTAAAAAGGCAATGATAGTAAGAAATGCT
AAAGATACAGCTCATACAAAAGCAGAGCGGAATATTCTGGAGGAAGT
AAAGCATCCCTTCATTGTGGATTTAATTTATGCCTTTCAGACCGGTG
GAAAACTCTACCTCATCCTTGAGTATCTCAGTGGAGGAGAACTATTT
ATGCAGTTAGAAAGAGAGGGGATATTCATGGAAGATACAGCTTGCTT
TTACTTGGCTGAAATCTCCATGGCTTTGGGGCATTTACATCAAAAAG
GGATCATCTACAGAGACCTGAAGCCGGAGAACATCATGCTTAATCAC
CAAGGTCACGTGAAGCTGACAGACTTTGGACTATGCAAAGAATCTAT
TCATGATGGAACAGTCACGCACACATTTTGTGGAACAATAGAATACA
TGGCCCCTGAAATCTTGATGAGAAGCGGCCACAACCGTGCTGTGGAT
TGGTGGAGTTTGGGAGCATTAATGTATGACATGCTGACTGGAGCACC
TCCATTCACTGGGGAGAATAGAAAGAAAACAATTGACAAAATCCTCA
AATGTAAACTTAATTTGCCTCCCTACCTCACACAAGAAGCTCGAGAT
CTGCTTAAAAAGCTGCTGAAAAGAAATGCTGCTTCTCGTCTTGGAGC
TGGCCCTGGGGATGCTGGAGAAGTCCAAGCGCATCCATTTTTTAGAC
ACATTAACTGGGAAGAGCTTTTGGCTCGGAAGGTGGAGCCCCCCTTT
AAGCCTCTGTTGCAATCTGAAGAGGATGTGAGTCAGTTTGATTCAAA
GTTTACTCGTCAGACACCTGTTGACAGCCCCGATGACTCAACTCTCA
GTGAAAGTGCCAACCAGGTCTTTCTGGGTTTCGAATACGTGGCTCCA
TCTGTACTTGAAAGTtga