MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of active GSK3 alpha [101 - 483]

Enzyme description:- GSK3 alpha [101 – 483]

Clone number:- DU 1894

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

Purification method:- Cobalt Agarose

Calculated molecular mass:-
Monoisotopic 46, 358.73 daltons
Average Mass 46, 388.02 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.63

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 10 mM DTT, 10 mM Magnesium acetate

Substrate:-
Phospho GS [YRRAAVPPSPSLSRHSSPHQS(PO4)EDEEE]

Final concentration: 30 μM

Clone Data Sheet

GSK3 alpha [101 - 483]

Protein	GSK3 alpha [101 - 483]
Clone number	DU 1894
Species	Human
Accession number	NM_019884.3
Tags	N-terminal His6
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**TTVVATLGQGPERSQEVAYT DIKVIGNGSFGVVYQARLAETRELVAIKKVLQDKRFKNRELQIMRKLD HCNIVRLRYFFYSSGEKKDELYLNLVLEYVPETVYRVARHFTKAKLTI PILYVKVYMYQLFRSLAYIHSQGVCHRDIKPQNLLVDPDTAVLKLCDF GSAKQLVRGEPNVSYICSRYYRAPELIFGATDYTSSIDVWSAGCVLAE LLLGQPIFPGDSGVDQLVEIIKVLGTPTREQIREMNPNYTEFKFPQIK AHPWTKVFKSRTPPEAIALCSSLLEYTPSSRLSPLEACAHSFFDELRC LGTQLPNNRPLPPLFNFSAGELSIQPSLNAILIPPHLRSPSGTTTLTP SSQALTETPTSSDWQSTDATPTLTNSS**
Native sequence	Amino acids M117 – E479 (end) of human PKB gamma. Residue T29 of the fusion protein is equivalent to T101 of the native enzyme. The His6 tag is located at residues 5 – 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 – 24
Cloning sites	*Bam*H1 and *Not*1 sites into pFastBac HTb

Nucleotide sequence of insert

ggatccACCACAGTCGTAGCCACTCTAGGCCAAGGCCCAGAGCGCTCC
CAAGAAGTGGCTTACACGGACATCAAAGTGATTGGCAATGGCTCATTT
GGGGTCGTGTACCAGGCACGGCTGGCAGAGACCAGGGAACTAGTCGCC
ATCAAGAAGGTTCTCCAGGACAAGAGGTTCAAGAACCGAGAGCTGCAG
ATCATGCGTAAGCTGGACCACTGCAATATTGTGAGGCTGAGATACTTT
TTCTACTCCAGTGGCGAGAAGAAAGACGAGCTTTACCTAAATCTGGTG
CTGGAATATGTGCCCGAGACAGTGTACCGGGTGGCCCGCCACTTCACC
AAGGCCAAGTTGACCATCCCTATCCTCTATGTCAAGGTGTACATGTAC
CAGCTCTTCCGCAGCTTGGCCTACATCCACTCCCAGGGCGTGTGTCAC
CGCGACATCAAGCCCCAGAACCTGCTGGTGGACCCTGACACTGCTGTC
CTCAAGCTCTGCGATTTTGGCAGTGCAAAGCAGTTGGTCCGAGGGGAG
CCCAATGTCTCCTACATCTGTTCTCGCTACTACCGGGCCCCAGAGCTC
ATCTTTGGAGCCACTGATTACACCTCATCCATCGATGTTTGGTCAGCT
GGCTGTGTACTGGCAGAGCTCCTCTTGGGCCAGCCCATCTTCCCTGGG
GACAGTGGGGTGGACCAGCTGGTGGAGATCATCAAGGTGCTGGGAACA
CCAACCCGGGAACAAATCCGAGAGATGAACCCCAACTACACGGAGTTC
AAGTTCCCTCAGATTAAAGCTCACCCCTGGACAAAGGTGTTCAAATCT
CGAACGCCGCCAGAGGCCATCGCGCTCTGCTCTAGCCTGCTGGAGTAC
ACCCCATCCTCAAGGCTCTCCCCACTAGAGGCCTGTGCGCACAGCTTC
TTTGATGAACTGCGATGTCTGGGAACCCAGCTGCCTAACAACCGCCCA
CTTCCCCCTCTCTTCAACTTCAGTGCTGGTGAACTCTCCATCCAACCG
TCTCTCAACGCCATTCTCATCCCTCCTCACTTGAGGTCCCCCAGCGGC
ACTACCACCCTCACCCCGTCCTCACAAGCTTTAACTGAGACTCCGACC
AGCTCAGACTGGCAGTCGACCGATGCCACACCTACCCTCACTAACTCC
TCCtgagcggccgc