

Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active GSK3 beta [2 - 420]

<u>Enzyme description:-</u>	GSK3 beta [2 - 420]
<u>Clone number:-</u>	DU 899
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6) and EE
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	3-5 mg/L
<u>Calculated molecular mass:-</u>	51, 020 daltons
<u>Purity:-</u>	>85 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	50 mM Tris-HCl pH 7.5, 50 % glycerol, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.
<u>Storage temperature:-</u>	-20 °C
<u>Assay:-</u>	Standard filter binding assay
<u>Assay buffer:-</u>	50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1mM EGTA, 10 mM MgAc
<u>Substrate:-</u>	Phospho GS [YRRAAVPPSPSLSRHSSPHQS(PO4)EDEEE] Final concentration: 30 μM
<u>Specific activity range:-</u>	200 – 400 U/mg

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Clone Data Sheet

GSK3 beta [2 - 420]

Protein GSK3 beta [2 - 420]

Clone number DU 899

Species Human

Accession no L33801

Tags N-terminal His(6) and EE (EFMPME)

Baculovirus expressed protein
MSYYHHHHHDYDIPPTENLYFQGAMGSATMEFMPME**SGRPRTTSFAE**
SCKPVQQPSAFGSMKVSRDKDGSKVTTVVATPGQGPDRPQEVSYTDTK
VIGNGSFGVYYQAKLCDSGELVAIKKVLQDKRFKNRELQIMRKLDHCN
IVRLRYFFYSSGEKKDEVYLNVLVDYVPETVYRVARHYSRAKQTLPI
YVKLYMYQLFRSLAYIHSFGICHREDIKPQNLLLPDTAVLKLCDFGSA
KQLVRGEPNVSYICSRYYRAPELIFGATDYTSSIDVWSAGCVLAELL
GQPIFPGDSGVQLVEIIKVLGTPTREQIREMNPNYTEFKFPQIKAHP
WTKVFRPRTPPEAIALCSRLLEYPTARLTPLEACAHSSFFDELRDPNV
KLPNGRDTPALFNFTTQELSSNPPLATILIPPHARIQAAASTPTNATA
ASDANTGDRGQTNNAAASASASNST

Native Sequence Amino acids S2 – T420 (end) of human GSK3 beta.

Residue S38 of the fusion protein is equivalent to S2 of the native enzyme. The His(6) tag is located at residues 5 - 10 and the EE tag at residues 32 - 37.

The following amino acid substitution is present:

H – **L**, where H350 of the native sequence is **L386** of the fusion protein

Protease cleavage rTEV (ENLYFQG) residues 18 - 24

Cloning sites *Bam*H1/*Bgl*II and *Not*1 of pFastBAC HTb

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Complete nucleotide sequence

ATGTCGTACTACCATTACCATCACCATCACGATTACGATATCCCAACG
ACCGAAAACCTGTATTTCAGGGGCCATGGGATCTGCCACCATGGAG
TTCATGCCATGGAGTCAGGGCGGCCAGAACCAACCTCCTTGCGGAG
AGCTGCAAGCCGGTGCAGCAGCCTCAGCTTGGCAGCATGAAAGTT
AGCAGAGACAAGGACGGCAGCAAGGTGACAACAGTGGTGGCAACTCCT
GGGCAGGGTCCAGACAGGCCACAAGAAGTCAGCTATAACAGACACTAA
GTGATTGAAATGGATCATTGGTGTGGTATATCAAGCAAACCTTGT
GATTCAAGGAGAACTGGTCGCCATCAAGAAAGTATTGCAGGACAAGAGA
TTTAAGAATCGAGAGCTCCAGATCATGAGAAAGCTAGATCACTGTAAC
ATAGTCGATTGCGTTATTCTTCTACTCCAGTGGTGAGAAGAAAGAT
GAGGTCTATCTTAATCTGGTGTGGACTATGTTCCGAAACAGTATAAC
AGAGTTGCCAGACACTATAGTCGAGCCAAACAGACGCTCCCTGTGATT
TATGTCAGTTGTATATGTATCAGCTGTTCCGAAGTTAGCCTATATC
CATTCTTGGAAATCTGCCATCGGGATATTAAACCGCAGAACCTCTTG
TTGGATCCTGATACTGCTGTATTAAAACCTGTGACTTGGAAAGTGCA
AAGCAGCTGGTCCGAGGAGAACCCAATGTTCGTATATCTGTTCTCGG
TACTATAGGGCACCAGAGTTGATCTTGGAGCCACTGATTACCTCT
AGTATAGATGTATGGTCTGCTGGCTGTGTGGCTGAGCTGTTACTA
GGACAACCAATATTCCAGGGGATAGTGGTGTGGATCAGTTGGTAGAA
ATAATCAAGGTCTGGAACTCCAACAAGGGAGCAAATCAGAGAAATG
AACCCAAACTACACAGAATTAAATTCCCTCAAATTAGGCACATCCT
TGGACTAAGGTCTCCGACCCGAACCTCCACCGGAGGCAATTGCACTG
TGTAGCCGTCTGCTGGAGTATACACCAACTGCCGACTAACACCACTG
GAAGCTTGTGCACATTCACTTTGATGAATTACGGGACCCAAATGTC
AAACTACCAATGGCGAGACACACCTGCACTCTCAACTTCACCACT
CAAGAACTGTCAAGTAATCCACCTCTGGCTACCATCCTTATTCTCCT
CATGCTCGGATTCAAGCAGCTGCTCAACCCCCACAAATGCCACAGCA
GCGTCAGATGCTAATACTGGAGACCGTGGACAGACCAATAATGCTGCT
TCTGCATCAGCTCCAACCTCCACCTgta