University of Dundee

Standard Operating Procedure

Preparation of active GSK3β

Enzyme description:-   Active GSK3β

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 with fresh baculovirus

Molecular mass:-   50 kDa by SDS-PAGE

Purity:-   >85%

Contaminants:-   The preparation contains several minor degradation products.

Activation protocol:-   N/A – constitutively active when purified from insect cells.

Enzyme storage buffer:-

50 mM Tris/HCl pH 7.5, 50% glycerol, 150 mM NaCl, 0.1 mM EGTA, 0.1 % β-mercaptoethanol, 0.02% Brij-35, 0.2 mM PMSF,1 mM Benzamidine.

Storage temperature:-   -20°C.

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CLONE DATA SHEET –human GSK3β

Protein	GSK3β
Accession no	L33801 human
Tags	His(6) and EE (EFMPME) amino-terminal
Baculovirus-expressed protein	MAHHHHHHGSATMEFMPME**SGRPRTTSFAESCKPVQQPSA FGSMKVSRDKDGSKVTTVVATPGQGPDRPQEVSYTDTKVI GNGSFGVVYQAKLCDSGELVAIKKVLQDKRFKNRELQIMR KLDHCNIVRLRYFFYSSGEKKDEVYLNLVLDYVPETVYRVA RHYSRAKQTLPVIYVKLYMYQLFRSLAYIHSFGICHRDIKPQ NLLLDPDTAVLKLCDFGSAKQLVRGEPNVSYICSRYYRAPEL IFGATDYTSSIDVWSAGCVLAELLLGQPIFPGDSGVDQLVEII KVLGTPTREQIREMNPNYTEFKFPQIKAHPWTKVFRPRTPPE AIALCSRLLEYTPTARLTPLEACAHSFFDELRDPNVKLPNGR DTPALFNFTTQELSSNPPLATILIPPHARIQAAASTPTNATAA SDANTGDRGQTNNAASASASNST**
Native Sequence	Residue 20 of the His$_6$-tagged protein is equivalent to Ser 2 of GSK3β. There is a His(6) tag at residues 3-8 and and EE (EFMPME) tag at residues 14-19.
Protease cleavage site	none
ORF in baculovirus	TCAGGGCGGCCCAGAACCACCTCCTTTGCGGAGAGCTGCAAGCCGGTG CAGCAGCCTTCAGCTTTTGGCAGCATGAAAGTTAGCAGAGACAAGGAC GGCAGCAAGGTGACAACAGTGGTGGCAACTCCTGGGCAGGGTCCAGAC AGGCCACAAGAAGTCAGCTATACAGACACTAAAGTGATTGGAAATGGA TCATTTGGTGTGGTATATCAAGCCAAACTTTGTGATTCAGGAGAACTG GTCGCCATCAAGAAAGTATTGCAGGACAAGAGATTTAAGAATCGAGAG CTCCAGATCATGAGAAAGCTAGATCACTGTAACATAGTCCGATTGCGT TATTTCTTCTACTCCAGTGGTGAGAAGAAAGATGAGGTCTATCTTAAT CTGGTGCTGGACTATGTTCCGGAAACAGTATACAGAGTTGCCAGACAC TATAGTCGAGCCAAACAGACGCTCCCTGTGATTTATGTCAAGTTGTAT ATGTATCAGCTGTTCCGAAGTTTAGCCTATATCCATTCCTTTGGAATC TGCCATCGGGATATTAAACCGCAGAACCTCTTGTTGGATCCTGATACT GCTGTATTAAAACTCTGTGACTTTGGAAGTGCAAAGCAGCTGGTCCGA

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GGAGAACCCAATGTTTCGTATATCTGTTCTCGGTACTATAGGGCACCA
GAGTTGATCTTTGGAGCCACTGATTATACCTCTAGTATAGATGTATGG
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AACTCCACCtga
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