

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Glycogenin [3 - 333]

Enzyme description:- Glycogenin [3 – 333]

Clone number:- DU 952

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 64, 100.30 daltons

Average Mass 64, 141.46 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.40

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

Glycogenin [3 – 333]

<u>Protein</u>	Glycogenin [3 – 333]
<u>Clone number</u>	DU 952
<u>Species</u>	Human
<u>Accession number</u>	AAB00114.1
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPKSDLEVL FQG PLGSADQAFVTLTTNDAY AKGALVVGSSLKQHRTRRLVVLATPQVSDSMRKVLETVFDEVIMVDVL DSGSAHLTLMKRPELGVTTLKLHCWSLTQYSKCVFMDADTLVLANIDD LFDREELSAAPDPGWPCFN SGVFVYQPSVETYNQLLHLASEQGSFDGG DQGILNTFFSSWATTDIRKHLFFIYNLSSISIYSYLP AFKVF GASAKVV HFLGRVKPWN YTYDPKTKSVKSEAHDPMTHPEFLILWWNI FTTNV LPL LQQFGLVKDTCSYVNVEDVSGAISHLSLGEIPAMAQPFVSSEERKERWE QGQADYMGADSF DN I KRKLD TYLQ</p>
<u>Native sequence</u>	<p>Amino acids D3 – Q333 (end) of human Glycogenin. Residue D233 of the fusion protein is equivalent to D3 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Eco</i> R1 sites of pGEX6P-1

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Nucleotide
Sequence Of
Insert

ggatccGCAGATCAGGCCTTTGTGACACTAACCACAAACGATGCCTACG
CCAAAGGTGCCCTGGTCCTGGGATCATCTCTGAAACAGCACAGGACCAC
CAGGAGGCTGGTCGTGCTCGCCACCCCTCAGGTCTCAGACTCCATGAGA
AAAGTTTTAGAGACAGTCTTTGATGAAGTCATCATGGTAGATGTCTTGG
ACAGTGGCGATTCTGCTCATCTAACCTTAATGAAGAGGCCAGAGTTGGG
TGTCACGCTGACAAAGCTCCACTGCTGGTCGCTTACACAGTATTCAAAA
TGTGTATTCATGGATGCAGATACTCTGGTCCTAGCAAATATTGATGATC
TTTTTGACAGAGAAGAATTGTCAGCAGCACCAGACCCAGGGTGGCCTGA
CTGCTTCAATTCCGGAGTCTTCGTTTTATCAGCCTTCAGTTGAAACATAC
AATCAGCTGTTGCATCTTGCTTCTGAGCAAGGTAGTTTTGATGGTGGGG
ACCAAGGCATACTGAACACATTTTTTTAGCAGCTGGGCAACAACAGATAT
CAGAAAACACCTGCCGTTTATTTATAACCTAAGCAGCATCTCTATATAC
TCCTACCTCCCGCATTTAAAGTGTTTGGTGCAAGTGCCAAAGTTGTGC
ATTCCTGGGACGAGTCAAACCATGGAATTATACTTATGATCCCAAAAC
AAAAAGTGTCAAAAGTGAGGCCCATGATCCCAACATGACTCATCCAGAG
TTTCTCATCCTGTGGTGGAACATCTTTACCACCAACGTTTTACCTCTGC
TTCAACAATTTGGCCTTGTCAAAGACACCTGCTCATATGTAAATGTGGA
AGATGTCTCAGGAGCCATATCACATCTGTCCCTTGGGGAGATCCCAGCT
ATGGCACAGCCGTTTGTATCCTCGGAAGAACGGAAGGAACGATGGGAAC
AGGGCCAGGCTGATTATATGGGAGCAGATTCCTTTGACAACATCAAGAG
GAAACTTGACACTTACCTCCAGtaggaattc