Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of c-MYC [2 – 439]

Enzyme description:- c-MYC [2 – 439]

Clone number:- DU 818

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST and His6

Purification method:- GSH Sepharose

Expression level:- 5 mg/L

Calculated molecular mass:-
Monoisotopic 76, 271.90 daltons
Average Mass 76, 319.90 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.68

Purity:- 70 %

Activation protocol:- Constitutively active

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:- -80 °C

Assay:- Substrate for GSK3

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Clone Data Sheet

c-MYC [2 – 439]

Protein	c-MYC [2 – 439]
Clone number	DU 818
Species	Human
Accession number	NM_008351
Tags	N-terminal GST and C-terminal His(6)
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**PLNVSFTNRNYDLD** **YDSVQPYFYCDEEENFYQQQQQSELQPPAPSEDIWKKFELLPTPPLSPS** **RRSGLCSPSYVAVTPFSLRGDNDGGGGSFSTADQLEMVTELLGGDMVNQ** **SFICDPDDETFIKNIIIQDCMWSGFSAAAKLVSEKLASYQAARKDSGSP** **NPARGHSVCSTSSLYLQDLSAAASECIDPSVVFPYPLNDSSSPKSCASQ** **DSSAFSPSSDSLLSSTESSPQGSPEPLVLHEETPPTTSSDSEEEQEDEE** **EIDVVSVEKRQAPGKRSESGSPSAGGHSKPPHSPLVLKRCHVSTHQHNY** **AAPPSTRKDYPAAKRVKLDSVRVLRQISNNRKCTSPRSSDTEENVKRRT** **HNVLERQRRNELKRSFFALRDQIPELENNEKAPKVVILKKATAYILSVQ** **AEEQKLISEEDLLRKRREQLKHKLEQLRNSCA**HHHHHH
Native sequence	Amino acids P2 – A439 (end) of human c-MYC. Residue P232 of the fusion protein is equivalent to P2 of the native enzyme. The GST tag is located at residues 1 – 220 and His6 tag at residues 670 – 675.
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Bam*HI and *Eco*R1site of pGEX 6P-1

Nucleotide Sequence of insert

ggatccCCCCTCAACGTTAGCTTCACCAACAGGAACTATGACCTCGACT
ACGACTCGGTGCAGCCGTATTTCTACTGCGACGAGGAGGAGAACTTCTA
CCAGCAGCAGCAGCAGAGCGAGCTGCAGCCCCCGGCGCCCAGCGAGGAT
ATCTGGAAGAAATTCGAGCTGCTGCCCACCCCGCCCCTGTCCCCTAGCC
GCCGCTCCGGGCTCTGCTCGCCCTCCTACGTTGCGGTCACACCCTTCTC
CCTTCGGGGAGACAACGACGGCGGTGGCGGGAGCTTCTCCACGGCCGAC
CAGCTGGAGATGGTGACCGAGCTGCTGGGAGGAGACATGGTGAACCAGA
GTTTCATCTGCGACCCGGACGACGAGACCTTCATCAAAAACATCATCAT
CCAGGACTGTATGTGGAGCGGCTTCTCGGCCGCCGCCAAGCTCGTCTCA
GAGAAGCTGGCCTCCTACCAGGCTGCGCGCAAAGACAGCGGCAGCCCGA
ACCCCGCCCGCGGCCACAGCGTCTGCTCCACCTCCAGCTTGTACCTGCA
GGATCTGAGCGCCGCCGCCTCAGAGTGCATCGACCCCTCGGTGGTCTTC
CCCTACCCTCTCAACGACAGCAGCTCGCCCAAGTCCTGCGCCTCGCAAG
ACTCCAGCGCCTTCTCTCCGTCCTCGGATTCTCTGCTGTCCTCGACGGA
GTCCTCCCCGCAGGGCAGCCCCGAGCCCCTGGTGCTCCATGAGGAGACA
CCGCCCACCACCAGCAGCGACTCTGAGGAGGAACAAGAAGATGAGGAAG
AAATCGATGTTGTTTCTGTGGAAAAGAGGCAGGCTCCTGGCAAAAGGTC
AGAGTCTGGATCACCTTCTGCTGGAGGCCACAGCAAACCTCCTCACAGC
CCACTGGTCCTCAAGAGGTGCCACGTCTCCACACATCAGCACAACTACG
CAGCGCCTCCCTCCACTCGGAAGGACTATCCTGCTGCCAAGAGGGTCAA
GTTGGACAGTGTCAGAGTCCTGAGACAGATCAGCAACAACCGAAAATGC
ACCAGCCCCAGGTCCTCGGACACCGAGGAGAATGTCAAGAGGCGAACAC
ACAACGTCTTGGAGCGCCAGAGGAGGAACGAGCTAAAACGGAGCTTTTT
TGCCCTGCGTGACCAGATCCCGGAGTTGGAAAACAATGAAAAGGCCCCC
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CAGAGGAGCAAAAGCTCATTTCTGAAGAGGACTTGTTGCGGAAACGACG
AGAACAGTTGAAACACAAACTTGAACAGCTACGGAACTCTTGTGCGCAC
CATCACCATCACCATtgagaattcgc