Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of NDRG1 [2 – 394]

Enzyme description:- NDRG1 [2 – 394]

Clone number:- DU 1557

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST and FLAG

Purification method:- GSH Sepharose

Expression level:- 5 mg/L

Calculated molecular mass:-
Monoisotopic 70, 780.83 daltons
Average Mass 70, 826.76 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.43

Purity:- >80 %

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 SGK and GSK3

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Clone Data Sheet

NDRG1 [2 – 394]

Protein	NDRG1 [2 – 394]
Clone number	DU 1557
Species	Human
Accession number	NM_006096
Tags	N-terminal GST and FLAG [DYKDDDDK]
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSATMDYKDDDDK**SRE** **MQDVDLAEVKPLVEKGETITGLLQEFDVQEQDIETLHGSVHVTLCGTPK** **GNRPVILTYHDIGMNHKTCYNPLFNYEDMQEITQHFAVCHVDAPGQQDG** **AASFPAGYMYPSMDQLAEMLPGVLQQFGLKSIIGMGTGAGAYILTRFAL** **NNPEMVEGLVLINVNPCAEGWMDWAASKISGWTQALPDMVVSHLFGKEE** **MQSNVEVVHTYRQHIVNDMNPGNLHLFINAYNSRRDLEIERPMPGTHTV** **TLQCPALLVVGDSSPAVDAVVECNSKLDPTKTTLLKMADCGGLPQISQP** **AKLAEAFKYFVQGMGYMPSASMTRLMRSRTASGSSVTSLDGTRSRSHTS** **EGTRSRSHTSEGTRSRSHTSEGAHLDITPNSGAAGNSAGPKSMEVSC**
Native sequence	Amino acids S2 – C394 (end) of human NDRG1. Residue S243 of the fusion protein is equivalent to S2 of the native enzyme. The GST tag is located at residues 1 – 220 and the FLAG tag is located at residues 235 – 242.
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Bam*HI sites of pGEX 6P-1

Nucleotide Sequence of insert

ggatccgccaccatggactacaaggacgacgatgacaagTCTCGGGAGA
TGCAGGATGTAGACCTCGCTGAGGTGAAGCCTTTGGTGGAGAAAGGGGA
GACCATCACCGGCCTCCTGCAAGAGTTTGATGTCCAGGAGCAGGACATC
GAGACTTTACATGGCTCTGTTCACGTCACGCTGTGTGGGACTCCCAAGG
GAAACCGGCCTGTCATCCTCACCTACCATGACATCGGCATGAACCACAA
AACCTGCTACAACCCCCTCTTCAACTACGAGGACATGCAGGAGATCACC
CAGCACTTTGCCGTCTGCCACGTGGACGCCCCTGGCCAGCAGGACGGCG
CAGCCTCCTTCCCCGCAGGGTACATGTACCCCTCCATGGATCAGCTGGC
TGAAATGCTTCCTGGAGTCCTTCAACAGTTTGGGCTGAAAAGCATTATT
GGCATGGGAACAGGAGCAGGCGCCTACATCCTAACTCGATTTGCTCTAA
ACAACCCTGAGATGGTGGAGGGCCTTGTCCTTATCAACGTGAACCCTTG
TGCGGAAGGCTGGATGGACTGGGCCGCCTCCAAGATCTCAGGATGGACC
CAAGCTCTGCCGGACATGGTGGTGTCCCACCTTTTTGGGAAGGAAGAAA
TGCAGAGTAACGTGGAAGTGGTCCACACCTACCGCCAGCACATTGTGAA
TGACATGAACCCCGGCAACCTGCACCTGTTCATCAATGCCTACAACAGC
CGGCGCGACCTGGAGATTGAGCGACCAATGCCGGGAACCCACACAGTCA
CCCTGCAGTGCCCTGCTCTGTTGGTGGTTGGGGACAGCTCGCCTGCAGT
GGATGCCGTGGTGGAGTGCAACTCAAAATTGGACCCAACAAAGACCACT
CTCCTCAAGATGGCGGACTGTGGCGGCCTCCCGCAGATCTCCCAGCCGG
CCAAGCTCGCTGAGGCCTTCAAGTACTTCGTGCAGGGCATGGGATACAT
GCCCTCGGCTAGCATGACCCGCCTGATGCGGTCCCGCACAGCCTCTGGT
TCCAGCGTCACTTCTCTGGATGGCACCCGCAGCCGCTCCCACACCAGCG
AGGGCACCCGAAGCCGCTCCCACACCAGCGAGGGCACCCGCAGCCGCTC
GCACACCAGCGAGGGGGCCCACCTGGACATCACCCCCAACTCGGGTGCT
GCTGGGAACAGCGCCGGGCCCAAGTCCATGGAGGTCTCCTGCtagggat
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