Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of NDRG2 [2 – 371]

Enzyme description:- NDRG2 [2 – 371]

Clone number:- DU 563

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST and FLAG

Purification method:- GSH Sepharose

Expression level:- 3 mg/L

Calculated molecular mass:-
Monoisotopic 69, 117.47 daltons
Average Mass 69, 161.91 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.19

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

NDRG2 [2 – 371]

Protein	NDRG2 [2 – 371]
Clone number	DU 563
Species	Human
Accession number	AAL08624
Tags	N-terminal GST and FLAG [DYKDDDDK]
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEFATMDYKDDDDK **AELQEVQITEEKPLLPGQTPEAAKEAELAARILLDQGQTHSVETPYGSV TFTAYGTPKPKRPAILTYHDVGLNYKSCFQPLFQFEDMQEIIQNFVRVH VDAPGMEEGAPVFPLGYQYPSLDQLADMIPCVLQYLNFSTIIGVGVGAG AYILARYALNHPDTVEGLVLINIDPNAKGWMDWAAHKLTGLTSSIPEMI LGHLFSQEELSGNSELIQKYRNIITHAPNLDNIELYWNSYNNRRDLNFE RGGNITLRCPVMLVVGDQAPHEDAVVECNSKLDPTQTSFLKMADSGGQP QLTQPGKLTEVFKYFLQGMGYMASSCMTRLSRSRTASLTSAASVDGNRS RSRTLSQSSESGTLSSGPPGHTMEVSC**
Native sequence	Amino acids A2 – C371 (end) of human NDRG2. Residue A246 of the fusion protein is equivalent to A2 of the native enzyme. The GST tag is located at residues 1 – 220 and the FLAG tag is located at residues 238 – 245.
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Eco*RI sites of pGEX 6P-1

Nucleotide Sequence of insert

gaattcgccaccatggactacaaggacgacgatgacaagGCGGAGCTGC
AGGAGGTGCAGATCACAGAGGAGAAGCCACTGTTGCCAGGACAGACGCC
TGAGGCGGCCAAGGAGGCTGAGTTAGCTGCCCGAATCCTCCTGGACCAG
GGACAGACTCACTCTGTGGAGACACCATACGGCTCTGTCACTTTCACTG
CCTATGGCACCCCCAAACCCAAACGCCCTGCGATCCTTACCTACCACGA
TGTGGGACTCAACTATAAATCTTGCTTCCAGCCACTGTTTCAGTTCGAG
GACATGCAGGAAATCATTCAGAACTTTGTGCGGGTTCATGTGGATGCCC
CTGGAATGGAAGAGGGAGCCCCTGTGTTCCCTTTGGGATATCAGTACCC
ATCTCTGGACCAGCTTGCGGACATGATCCCTTGCGTCCTGCAGTACCTA
AATTTCTCTACAATAATTGGAGTTGGTGTTGGAGCTGGAGCCTACATCC
TGGCGAGATATGCTCTTAACCACCCGGACACTGTTGAAGGTCTTGTCCT
CATCAACATTGATCCCAATGCCAAGGGTTGGATGGATTGGGCAGCCCAC
AAGCTAACAGGCCTCACCTCTTCCATTCCGGAGATGATCCTTGGACATC
TTTTCAGCCAGGAAGAGCTCTCTGGAAATTCTGAGTTGATACAAAAGTA
CAGAAATATCATTACACATGCACCCAACCTGGATAACATTGAATTGTAC
TGGAACAGCTACAACAACCGCCGAGACCTGAACTTTGAGCGTGGAGGTA
ATATCACCCTCAGGTGCCCTGTGATGCTGGTGGTAGGAGACCAAGCACC
TCATGAAGATGCAGTGGTGGAATGTAACTCAAAACTGGATCCCACCCAG
ACCTCGTTCCTCAAAATGGCTGACTCCGGAGGTCAGCCCCAGCTGACTC
AGCCAGGCAAGCTGACCGAGGTCTTCAAGTACTTCCTGCAAGGCATGGG
CTACATGGCCTCATCCTGCATGACTCGCCTGTCCCGGTCTCGTACAGCC
TCTCTGACCAGTGCAGCATCCGTTGATGGCAACCGGTCCCGCTCTCGCA
CCCTGTCCCAGAGCAGCGAGTCTGGAACTCTTTCTTCGGGGCCCCCGGG
GCACACCATGGAGGTCTCCTGTtgagaattc