Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active DDR2 [467 - 854]

Enzyme description:- DDR2 [467 - 854]

Clone number:- DU 32877

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- Glutathione Sepharose

Calculated molecular mass:-
Monoisotopic 71, 847.16 daltons
Average Mass 71, 893.44 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.57

Purity:- >80 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF.

Storage temperature:- -70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2–mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-
KKSRGDYMTMQIG Final concentration: 300 μM

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Clone Data Sheet

DDR2 [467 - 854]

Protein	DDR2 [467 - 854]
Clone number	DU 32877
Species	Human
Accession number	NM_006182
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPGIPGSTRAAA**SNS** **TYDRIFPLRPDYQEPSRLIRKLPEFAPGEEESGCSGVVKPVQPSGPEGV** **PHYAEADIVNLQGVTGGNTYSVPAVTMDLLSGKDVAVEEFPRKLLTFKE** **KLGEGQFGEVHLCEVEGMEKFKDKDFALDVSANQPVLVAVKMLRADANK** **NARNDFLKEIKIMSRLKDPNIIHLLAVCITDDPLCMITEYMENGDLNQF** **LSRHEPPNSSSSDVRTVSYTNLKFMATQIASGMKYLSSLNFVHRDLATR** **NCLVGKNYTIKIADFGMSRNLYSGDYYRIQGRAVLPIRWMSWESILLGK** **FTTASDVWAFGVTLWETFTFCQEQPYSQLSDEQVIENTGEFFRDQGRQT** **YLPQPAICPDSVYKLMLSCWRRDTKNRPSFQEIHLLLLQQGDE**
Native sequence	Amino acids S467 – E854 (end) of human DDR2. Residue E243 of the fusion protein is equivalent to E467 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 229
Cloning sites	*Not*1 sites in pFastBAC GST

Nucleotide sequence of insert

gcggccgcgTCCAACTCGACTTACGATCGCATCTTTCCCCTTCGCCCTG
ACTACCAGGAGCCATCCAGGCTGATACGAAAACTCCCAGAATTTGCTCC
AGGGGAGGAGGAGTCAGGCTGCAGCGGTGTTGTGAAGCCAGTCCAGCCC
AGTGGCCCTGAGGGGGTGCCCCACTATGCAGAGGCTGACATAGTGAACC
TCCAAGGAGTGACAGGAGGCAACACATACTCAGTGCCTGCCGTCACCAT
GGACCTGCTCTCAGGAAAAGATGTGGCTGTGGAGGAGTTCCCCAGGAAA
CTCCTAACTTTCAAAGAGAAGCTGGGAGAAGGACAGTTTGGGGAGGTTC
ATCTCTGTGAAGTGGAGGGAATGGAAAAATTCAAAGACAAAGATTTTGC
CCTAGATGTCAGTGCCAACCAGCCTGTCCTGGTGGCTGTGAAAATGCTC
CGAGCAGATGCCAACAAGAATGCCAGGAATGATTTTCTTAAGGAGATAA
AGATCATGTCTCGGCTCAAGGACCCAAACATCATCCATCTATTAGCTGT
GTGTATCACTGATGACCCTCTCTGTATGATCACTGAATACATGGAGAAT
GGAGATCTCAATCAGTTTCTTTCCCGCCACGAGCCCCCTAATTCTTCCT
CCAGCGATGTACGCACTGTCAGTTACACCAATCTGAAGTTTATGGCTAC
CCAAATTGCCTCTGGCATGAAGTACCTTTCCTCTCTTAATTTTGTTCAC
CGAGATCTGGCCACACGAAACTGTTTAGTGGGTAAGAACTACACAATCA
AGATAGCTGACTTTGGAATGAGCAGGAACCTGTACAGTGGTGACTATTA
CCGGATCCAGGGCCGGGCAGTGCTCCCTATCCGCTGGATGTCTTGGGAG
AGTATCTTGCTGGGCAAGTTCACTACAGCAAGTGATGTGTGGGCCTTTG
GGGTTACTTTGTGGGAGACTTTCACCTTTTGTCAAGAACAGCCCTATTC
CCAGCTGTCAGATGAACAGGTTATTGAGAATACTGGAGAGTTCTTCCGA
GACCAAGGGAGGCAGACTTACCTCCCTCAACCAGCCATTTGTCCTGACT
CTGTGTATAAGCTGATGCTCAGCTGCTGGAGAAGAGATACGAAGAACCG
TCCCTCATTCCAAGAAATCCACCTTCTGCTCCTTCAACAAGGCGACGAG
tgagcggccgc