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

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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
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