Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active CARP2 [1 – 363]

Enzyme description:- CARP2 [1 – 363]

Clone number:- DU 43464

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 67, 148.85 daltons
Average Mass 67, 192.87 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.48

Purity:- 85 %

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, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- –70 °C

Assay:-
Ubiquitin chain assay with UBE1, Ubiquitin and E2 (UBE2D1, D2, D3 or UBE2N/UBDE2V1). Adding CARP2 will increase the amount if long isopeptide chains as monitored by immunoblotting against Ubiquitin.

Assay buffer:-
25 mM HEPES pH 7.5, 1 mM DTT, 10 mM Mg-acetate, 0.2 mM ATP

Assay conditions:-
2 hours at 30 - 37 °C

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Clone Data Sheet

CARP2 [1 – 363]

Protein	CARP2 [1 - 363]
Clone number	DU 43464
Species	Human
Accession number	NM_001017368
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERA EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFED RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDENLYF QGGS**MWATCCNWFCLDGQPEEVPPPQGARMQAYSNPGYSSFPSPT GLEPSCKSCGAHFANTARKQTCLDCKKNFCMTCSSQVGNGPRLCL LCQRFRATAFQREELMKMKVKDLRDYLSLHDISTEMCREKEELVL LVLGQQPVISQEDRTRASTLSPDFPEQQAFLTQPHSSMVPPTSPN LPSSSAQATSVPPAQVQENQQANGHVSQDQEEPVYLESVARVPAE DETQSIDSEDSFVPGRRASLSDLTDLEDIEGLTVRQLKEILARNF VNYKGCCEKWELMERVTRLYKDQKGLQHLVSGAEDQNGGAVPSGL EENLCKICMDSPIDCVLLECGHMVTCTKCGKRMNECPICRQYVIR AVHVFRS**
Native sequence	Amino acids M1 – S363 of human CARP2. Residue M230 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 - 220.
Protease cleavage	rTev (ENLYFQG) residues 221 - 227
Cloning sites	*Bam*H1 and *Not*1 sites of pGEX-6P-1

Nucleotide sequence of insert

ggatccATGTGGGCAACCTGCTGCAACTGGTTCTGCCTGGATGGA
CAGCCTGAGGAGGTCCCACCACCCCAGGGAGCCAGGATGCAGGCC
TATTCCAACCCTGGGTACAGCTCCTTCCCTTCCCCAACAGGCTTG
GAACCAAGCTGCAAGTCCTGTGGGGCTCACTTTGCAAACACGGCC
AGGAAGCAGACCTGCTTGGACTGTAAGAAAAATTTTTGCATGACC
TGTTCGAGCCAAGTAGGGAATGGGCCCCGCCTCTGCCTTCTCTGC
CAACGGTTTCGAGCTACAGCCTTTCAGCGAGAGGAGCTCATGAAG
ATGAAGGTGAAGGACTTGAGGGACTATCTCAGCCTCCATGACATC
TCTACCGAAATGTGCCGGGAGAAAGAAGAGCTGGTGCTCTTGGTC
CTTGGCCAGCAGCCTGTAATCTCCCAGGAGGACAGGACTCGTGCC
TCCACCTTGTCCCCAGACTTTCCTGAGCAGCAGGCCTTCCTGACC
CAGCCTCACTCCAGCATGGTTCCACCTACCTCACCCAACCTCCCC
TCTTCATCTGCACAAGCCACCTCTGTTCCCCCAGCCCAGGTTCAG
GAGAATCAGCAGGCCAATGGCCATGTGTCTCAGGATCAAGAGGAA
CCCGTCTACCTGGAGAGCGTGGCCAGAGTACCTGCTGAGGATGAG
ACCCAGTCTATTGACTCAGAGGACAGCTTTGTCCCAGGCCGAAGG
GCCTCTCTGTCTGACCTGACTGACCTGGAGGACATTGAAGGCCTG
ACAGTGCGGCAGCTGAAAGAGATCTTGGCTCGCAACTTTGTCAAC
TACAAGGGCTGCTGTGAGAAGTGGGAGCTGATGGAGAGAGTGACC
CGGCTATACAAGGATCAGAAAGGACTCCAGCACCTGGTCAGTGGT
GCCGAAGACCAAAACGGGGGAGCAGTACCATCAGGCTTGGAGGAG
AACCTGTGTAAGATCTGCATGGACTCACCCATTGACTGTGTTCTT
CTGGAGTGTGGCCACATGGTAACCTGTACCAAGTGTGGCAAGCGC
ATGAATGAATGTCCCATCTGCCGGCAGTATGTAATCCGAGCTGTG
CATGTCTTCCGGTCCtgagcggccgc