MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of 53BP1 [1 – 500]

Enzyme description:- 53BP1 [1 - 500]

Clone number:- DU 11191

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 80, 637.27 daltons
Average Mass 80, 687.58 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 4.51

Purity:- >85 %

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:- -70 °C

MRC PPU Reagents and Services

Clone Data Sheet

53BP1 [1 - 500]

Protein	53BP1[1 - 500]
Clone number	DU 11191
Species	Human
Accession number	NM_001141980.3
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MDPTGSQLDSDFSQ QDTPCLIIEDSQPESQVLEDDSGSHFSMLSRHLPNLQTHKENPVLDVVS NPEQTAGEERGDGNSGFNEHLKENKVADPVDSSNLDTCGSISQVIEQLP QPNRTSSVLGMSVESAPAVEEEKGEELEQKEKEKEEDTSGNTTHSLGAE DTASSQLGFGVLELSQSQDVEENTVPYEVDKEQLQSVTTNSGYTRLSDV DANTAIKHEEQSNEDIPIAEQSSKDIPVTAQPSKDVHVVKEQNPPPARS EDMPFSPKASVAAMEAKEQLSAQELMESGLQIQKSPEPEVLSTQEDLFD QSNKTVSSDGCSTPSREEGGCSLASTPATTLHLLQLSGQRSLVQDSLST NSSDLVAPSPDAFRSTPFIVPSSPTEQEGRQDKPMDTSVLSEEGGEPFQ KKLQSGEPVELENPPLLPESTVSPQASTPISQSTPVFPPGSLPIPSQPQ FSHDIFIPSPSLEEQSNDGKKDGDMHSSSLTVECSKTSEIEPKNS**
Native sequence	Amino acids M1 – S500 of human 53BP1 (end residue is H1972) Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 229
Cloning sites	*BamH1* and *Not*1 site of pGex6P-1

Nucleotide Sequence of Insert

ggatccATGGACCCTACTGGAAGTCAGTTGGATTCAGATTTCTCTCAGCAAGATACTCCTTGCCTG
ATAATTGAAGATTCTCAGCCTGAAAGCCAGGTTCTAGAGGATGATTCTGGTTCTCACTTCAGTATG
CTATCTCGACACCTTCCTAATCTCCAGACGCACAAAGAAAATCCTGTGTTGGATGTTGTGTCCAAT
CCTGAACAAACAGCTGGAGAAGAACGAGGAGACGGTAATAGTGGGTTCAATGAACATTTGAAAGAA
AACAAGGTTGCAGACCCTGTGGATTCTTCTAACTTGGACACATGTGGTTCCATCAGTCAGGTCATT
GAGCAGTTACCTCAGCCAAACAGGACAAGCAGTGTTCTGGGAATGTCAGTGGAATCTGCTCCTGCT
GTGGAGGAAGAGAAGGGAGAAGAGTTGGAACAGAAGGAGAAAGAGAAGGAAGAAGATACTTCAGGC
AATACTACACATTCCCTTGGTGCTGAAGATACTGCCTCATCACAGTTGGGTTTTGGGGTTCTGGAA
CTCTCCCAGAGCCAGGATGTTGAGGAAAATACTGTGCCATATGAAGTGGACAAAGAGCAGCTACAA
TCAGTAACCACCAACTCTGGTTATACCAGGCTGTCTGATGTGGATGCTAATACTGCAATTAAGCAT
GAAGAACAGTCCAACGAAGATATCCCCATAGCAGAACAGTCCAGCAAGGACATCCCTGTGACAGCA
CAGCCCAGTAAGGATGTACATGTTGTAAAAGAGCAAAATCCACCACCTGCAAGGTCAGAGGACATG
CCTTTTAGCCCCAAAGCATCTGTTGCTGCTATGGAAGCAAAAGAACAGTTGTCTGCACAAGAACTT
ATGGAAAGTGGACTGCAGATTCAGAAGTCACCAGAGCCTGAGGTTTTGTCAACTCAGGAAGACTTG
TTTGACCAGAGCAATAAAACAGTATCTTCTGATGGTTGCTCTACTCCTTCAAGGGAGGAAGGTGGG
TGTTCTTTGGCTTCCACTCCTGCCACCACTCTGCATCTCCTGCAGCTCTCTGGTCAGAGGTCCCTT
GTTCAGGACAGTCTTTCCACGAATTCTTCAGATCTTGTTGCTCCTTCTCCTGATGCTTTCCGATCT
ACTCCTTTTATCGTTCCTAGCAGTCCCACAGAGCAAGAAGGGAGACAAGATAAGCCAATGGACACG
TCAGTGTTATCTGAAGAAGGAGGAGAGCCTTTTCAGAAGAAACTTCAAAGTGGTGAACCAGTGGAG
TTAGAAAACCCCCCTCTCCTGCCTGAGTCCACTGTATCACCACAAGCCTCAACACCAATATCTCAG
AGCACACCAGTCTTCCCTCCTGGGTCACTTCCTATCCCATCCCAGCCTCAGTTTTCTCATGACATT
TTTATTCCTTCCCCAAGTCTGGAAGAACAATCAAATGATGGGAAGAAAGATGGAGATATGCATAGT
TCATCTTTGACAGTTGAGTGTTCTAAAACTTCAGAGATTGAACCAAAGAATTCCtag