

MRCPPU Reagents and Services

Standard Operating Procedure

Preparation of RAD52 [1 - 418]

Enzyme description:- RAD52 [1 - 418]

Clone number:- DU 67602

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH-Sepharose

Calculated molecular mass:-

Monoisotopic 72946.68 daltons

Average Mass 72992.82 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.99

Purity:- >80 %

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

MRCPPU Reagents and Services

Clone Data Sheet

RAD52 [1 - 418]

<u>Protein</u>	RAD52 [1 - 418]
<u>Clone number</u>	DU 67602
<u>Species</u>	Human
<u>Accession number</u>	P43351-1
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFOGPLGSMGTEEA ILGGRDS HPAAGGGSVLCFGQCQYTAEEYQAIQKALRQRLGPEYISSRMAGGGQKV CYIEGHRVINLANEMFGYNGWAHSITQQNVDFVDLNNGKFYVGCAFVR VQLKDGSYHEDVGYGVSEGLKSKALSLEKARKEAVTDGLKRALRSFGNA LGNCILDKDYLRSLNKLPRQLPLEVDLTKAKRQDLEPSVEEARYNCRP NMALGHPQLQOVTPSRP SHAVIPADQDCSSRSLSSSAVESEATHQRKL RQKQLQQQFRERMEKQQVRVSTPSAEKSEAAPPAPPVTHSTPVTVSEPL LEKDFLAGVTQELIKTLEDNSEKWAVTPDAGDG VVKPSSRADPAQTSDT LALNNQMV TQNRTPHSVCHQKPQAKSGSWDLQTY SADQRTTGNWESHK SQDMKKRKYDPS</p>
<u>Native sequence</u>	<p>Amino acids M1 – S418 (end residue) of human RAD52. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 – 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX6P-1

MRCPPU Reagents and Services

Nucleotide Sequence of Insert

ggatccATGTCTGGGACTGAGGAAGCAATTCTTGGAGGACGTGACAGCCATCCTGCTGCTGGCGGC
GGCTCAGTGTTATGCTTTGGACAGTGCCAGTACACAGCAGAAGAGTACCAGGCCATCCAGAAGGCC
CTGAGGCAGAGGCTGGGCCCAGAATACATAAGTAGCCGCATGGCTGGCGGAGGCCAGAAGGTGTGC
TACATTGAGGGTCATCGGGTAATTAATCTGGCCAATGAGATGTTTGGTTACAATGGCTGGGCACAC
TCCATCACGCAGCAGAATGTGGATTTTGTGACCTCAACAATGGCAAGTTCTACGTGGGAGTCTGT
GCATTTGTGAGGGTCCAGCTGAAGGATGGTTCATATCATGAAGATGTTGGTTATGGTGTTAGTGAG
GGCCTCAAGTCCAAGGCTTTTATCTTTGGAGAAGGCAAGGAAGGAGGCGGTGACAGACGGGCTGAAG
CGAGCCCTCAGGAGTTTGGGAATGCACTTGGAACTGTATTCTGGACAAAGACTACCTGAGATCA
CTAAATAAGCTTCCACGCCAGTTGCCTCTTGAAGTGGATTTAACTAAAGCGAAGAGACAAGATCTT
GAACCGTCTGTGGAGGAGGCAAGATAACAACAGCTGCCGACCGAACATGGCCCTGGGACACCCACAG
CTGCAGCAGGTGACCTCCCCTTCCAGACCCAGCCATGCTGTGATACCGGCGGACCAGGACTGCAGC
TCCCGAAGCCTGAGCTCATCCGCCGTGGAGAGCGAGGCCACGCACCAGCGGAAGCTCCGGCAGAAG
CAGCTGCAGCAGCAGTTCCGGGAGCGGATGGAGAAGCAGCAGGTTTCGAGTCTCCACGCCGTCAGCT
GAGAAGAGTGAGGCAGCGCTCCGGCCCCTCCTGTGACGCACAGCACTCCTGTAAGTGTCTCAGAA
CCACTCCTGGAGAAAGACTTCCCTGCAGGAGTGAAGTCAAGAATTAATCAAGACTCTTGAAGACAAC
TCTGAAAAGTGGGCTGTGACTCCCGATGCAGGGGATGGTGTGGTCAAGCCCTCGTCTAGAGCAGAC
CCAGCCCAGACCTCTGACACATTAGCCTTGAACAACCAGATGGTGACCCAGAACAGGACTCCACAC
AGCGTTTGGCACCAGAAACCACAAGCAAAATCTGGATCTTGGGACCTCCAAACTTATAGCGCTGAC
CAACGCACAACAGGAAACTGGGAATCTCATAGGAAGAGCCAGGACATGAAGAAAAGGAAATATGAT
CCATCTtaagcggccgc

MRCPPU Reagents and Services

MRCPPU Reagents and Services