

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of MCM2 [2 - 895]**

**Enzyme description:-** MCM2 [2 - 895]

**Clone number:-** DU 1046

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 127, 538.38 daltons

Average Mass 127, 619.05 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 5.45

**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

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**Clone Data Sheet**

**MCM2 [2 - 895]**

**Protein** MCM2 [2 - 895]

**Clone number** DU 1046

**Species** Human

**Accession number** D83987.1

**Tags** N-terminal GST

**Bacterially expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG  
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAESMLEGA  
VLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDH  
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS  
KYIAWPLQGWQATFGGGDHPPKSDLEVLVFGQPLGSASSPAQRRRGNDPL  
**TSSPGRSSRRTDALTSSPGRDLPPFEDESEGLLGTEGPLEEEEDGEELI**  
**GDGMERDYRAIPELDAYEA EGLALDDEDVEELTASQREAAERAMRQDR**  
**EAGRGLGRMRRGLLYDSDEEDEER PARKRRQVERATEDGEEDEEMIESI**  
**ENLEDLKGHSVREWVSMAGPRLEIHHRFKNFLRTHVDSHGHNVFKERIS**  
**DMCKENRESLVVNYEDLAAREHV LAYFLPEAPAELLQIFDEAALEV VLA**  
**MYPKYDRITNHIHVRI SHLPLVEELRSLRQLHLNQLIRTSQVVTSTCTGV**  
**LPQLSMVKYCNKCNFVLGPFQSQSQSQEQV KPGSCPECQSAGPFVNMEE**  
**TIYQNYQRI RIQESPGKVAAGRLPRSKDAILLADLVDSCKPGDEIELTG**  
**IYHNNYDGS LNTANGFPVFATVILANHVAKKDNKVAVGELTDEDVKMIT**  
**SLSKDQQIGEKIFASIAPSIYGHEDIKRGLALALFGGEPKNP GGKHKVR**  
**GDINVLLCGDPGTAKSQFLKYIEKVSSRAIFTTGQGASAVGLTAYVQRH**  
**PVSREWTLEAGALVLADRGVCLIDEFDKMNDQDRTSIHEAMEQQSISIS**  
**KAGIVTSLQARCTVIAAANPIGGRYDPSLTFSENVDLTEPIISRFDILC**  
**VVRDTVDPVQDEMLARFVVGSHVRHHPSNKEEEGLANGSAAEPAMPNTY**  
**GVEPLPQEV LKKYI IYAKERVHPKLNQMDQDKVAKMYSDLRKESMATGS**  
**IPITVRHIESMIRMAEAHARIHLRDYVIEDDVNMAIRVMLESFIDTQKF**  
**SVMRSMRKT FARYLSFRDNNELLF ILKQLVAEQVTYQRNRFGAQODT**  
**IEVPEKDLVDKARQINIHNL SAFYDSE LFRMNKFSHDLKRKMILOQF**

**Native sequence** Amino acids A2 – F895 (end) of human MCM2.  
Residue A232 of the fusion protein is equivalent to A2 of the native enzyme. The GST tag is located at residues 1 – 220.

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Protease cleavage      PreScission (LEVLFQGP) residues 221 - 228

Cloning sites            *Bam*H1 and *Eco*R1 of pGEX6P-1

### Nucleotide Sequence of Insert

ggatccGCATCCAGCCCGGCCAGCGTCGGCGAGGCAATGATCCTCTCAC  
CTCCAGCCCTGGCCGAAGCTCCCGGCGTACTGATGCCCTCACCTCCAGCC  
CTGGCCGTGACCTTCCACCATTTGAGGATGAGTCCGAGGGGCTCCTAGGC  
ACAGAGGGGGCCCTGGAGGAAGAAGAGGATGGAGAGGAGCTCATTTGGAGA  
TGGCATGGAAAGGACTACCGCGCCATCCAGAGCTGGACGCCTATGAGG  
CCGAGGGACTGGCTCTGGATGATGAGGACGTAGAGGAGCTGACGGCCAGT  
CAGAGGGAGGCAGCAGAGCGGGCCATGCGGCAGCGTGACCGGGAGGCTGG  
CCGGGGCCTGGGCCGCATGCGCCGTGGGCTCCTGTATGACAGCGATGAGG  
AGGACGAGGAGCGCCCTGCCCGCAAGCGCCGCCAGGTGGAGCGGGCCACG  
GAGGACGGCGAGGAGGACGAGGAGATGATCGAGAGCATCGAGAACCTGGA  
GGATCTCAAAGGCCACTCTGTGCGCGAGTGGGTGAGCATGGCGGGCCCC  
GGCTGGAGATCCACCACCGCTTCAAGAACTTCTGCGCACTCACGTGAC  
AGCCACGGCCACAACGTCTTCAAGGAGCGCATCAGCGACATGTGCAAAGA  
GAACCGTGAGAGCCTGGTGGTGAACATGAGGACTTGGCAGCCAGGGAGC  
ACGTGCTGGCCTACTTCTGCTGAGGCACCGGCGGAGCTGCTGCAGATC  
TTTGATGAGGCTGCCCTGGAGGTGGTACTGGCCATGTACCCCAAGTACGA  
CCGCATCACCAACCACATCCATGTCCGCATCTCCACCTGCCTCTGGTGG  
AGGAGCTGCGCTCGCTGAGGCAGCTGCATCTGAACCAGCTGATCCGCACC  
AGTGGGGTGGTGACCAGCTGCACTGGCGTCTGCCCCAGCTCAGCATGGT  
CAAGTACAACCTGCAACAAGTGAATTTCTGTCCTGGGTCTTTCTGCCAGT  
CCCAGAACCAGGAGGTGAAACCAGGCTCCTGTCTGAGTGCCAGTCGGCC  
GGCCCCTTTGAGGTCAACATGGAGGAGACCATCTATCAGAACTACCAGCG  
TATCCGAATCCAGGAGAGTCCAGGCAAAGTGGCGGCTGGCCGGCTGCCCC  
GCTCCAAGGACGCCATTTCTCCTCGCAGATCTGGTGGACAGCTGCAAGCCA  
GGAGACGAGATAGAGCTGACTGGCATCTATCACAACAACATGATGGCTC  
CCTCAACACTGCCAATGGCTTCCCTGTCTTTGCCACTGTCATCCTAGCCA  
ACCACGTGGCCAAGAAGGACAACAAGGTTGCTGTAGGGGAACTGACCGAT  
GAAGATGTGAAGATGATCACTAGCCTCTCCAAGGATCAGCAGATCGGAGA  
GAAGATCTTTGCCAGCATTGCTCCTTCCATCTATGGTCATGAAGACATCA  
AGAGAGGCCTGGCTCTGGCCCTGTTCCGAGGGGAGCCAAAAACCAGGT  
GGCAAGCACAAAGGTACGTGGTGATATCAACGTGCTCTTGTGCGGAGACCC  
TGGCACAGCGAAGTCGCAGTTTCTCAAGTATATTGAGAAAGTGTCCAGCC  
GAGCCATCTTACCCTGGCCAGGGGGCGTCGGCTGTGGGCCTCACGGCG  
TATGTCCAGCGGCACCCTGTGAGCAGGGAGTGGACCTTGGAGGCTGGGGC  
CCTGGTTCTGGCTGACCGAGGAGTGTGTCTCATTGATGAATTTGACAAGA  
TGAATGACCAGGACAGAACCAGCATCCATGAGGCCATGGAGCAACAGAGC  
ATCTCCATCTCGAAGGCTGGCATCGTCACCTCCCTGCAGGCTCGCTGCAC  
GGTCATTGCTGCCGCCAACCCCATAGGAGGGCGCTACGACCCCTCGCTGA  
CTTCTCTGAGAACGTGGACCTCACAGAGCCCATCATCTCACGCTTTGAC  
ATCCTGTGTGTGGTGAGGGACACCGTGGACCCAGTCCAGGACGAGATGCT  
GGCCCGCTTCGTGGTGGGCAGCCACGTCAGACACCACCCAGCAACAAGG  
AGGAGGAGGGGCTGGCCAATGGCAGCGCTGCTGAGCCCGCCATGCCAAC  
ACGTATGGCGTGGAGCCCCTGCCCGAGGAGGTCTGAAGAAGTACATCAT

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CTACGCCAAGGAGAGGGTCCACCCGAAGCTCAACCAGATGGACCAGGACA  
AGGTGGCCAAGATGTACAGTGACCTGAGGAAAGAATCTATGGCGACAGGC  
AGCATCCCCATTACGGTGCGGCACATCGAGTCCATGATCCGCATGGCGGA  
GGCCCACGCGCGCATCCATCTGCGGGACTATGTGATCGAAGACGACGTCA  
ACATGGCCATCCGCGTGATGCTGGAGAGCTTCATAGACACACAGAAGTTC  
AGCGTCATGCGCAGCATGCGCAAGACTTTTGCCCGCTACCTTTCATTCCG  
GCGTGACAACAATGAGCTGTTGCTCTTCATACTGAAGCAGTTAGTGGCAG  
AGCAGGTGACATATCAGCGCAACCGCTTTGGGGCCAGCAGGACACTATT  
GAGGTCCCTGAGAAGGACTTGGTGGATAAGGCTCGTCAGATCAACATCCA  
CAACCTCTCTGCATTTTATGACAGTGAGCTCTTCAGGATGAACAAGTTCA  
GCCACGACCTGAAAAGGAAAATGATCCTGCAGCAGTTctgagaattc

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agcatccccatta  
cggcgcgccacat  
cgagtcgatgatc  
cgcatggcgga  
ggcccacgcgcgc  
atccatctgcggg  
actatgtgatcga  
agacgacgtca  
acatggccatccg  
cgtgatgctggag  
agcttcatagaca  
cacagaagttc  
agcgtcatgcgca  
gcatgcgcaagac  
ttttgcccgtac  
ctttcattccg  
gcgtgacaacaat  
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tcatactgaagca  
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tcagcgcaaccgc  
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ctgcagcagttct  
gagaattc