*Division of Signal Tranduction Therapy*

# Standard Operating Procedure

# Preparation of MAPKAP-K2 D207A [46 – 400]

**Enzyme description:-** MAPKAP-K2 D207A [46 - 400]

**Clone number:-** DU 51273

**Source:-** Recombinant

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

**Tag:-** N-terminal GST and C-terminal Myc

**Purification method**:- GSH Sepharose

**Calculated molecular mass:-**
Monoisotopic 69, 978.92 daltons
Average Mass 70, 024.28 daltons
[cysteines reduced, methionines have not been oxidised

**Theoretical pI:-** 8.10

**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

## MAPKAP-K2 D207A [46 – 400]

| | |
|---|---|
| **Protein** | MAPKAP-K2 D207A [46 - 400] |
| **Clone number** | DU 51273 |
| **Species** | Human |
| **Accession number** | NM_032960 |
| **Tags** | N-terminal GST |
| **Bacterially expressed protein** | MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL<br>GLEFPNPLYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE<br>GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLHMFEDRLCHKTYLN<br>GDHVTHPDFMLYDALAVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY<br>LKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSPGISGGGGGILEAT<br>ME**FHVKSGLQIKKNAIIDDYKVTSQVLGLGINGKVLQIFNKRTQEKFA**<br>**LKMLQDCPKARREVELHWRASQCPHIVRIVDVYENLYAGRKCLLIVME**<br>**CLDGGELFSRIQDRGDQAFTEREASEIMKSIGEAIQYLHSINIAHRDV**<br>**KPENLLYTSKRPNAILKLTAFGFAKETTSHNSLTTPCYTPYYVAPEVL**<br>**GPEKYDKSCDMWSLGVIMYILLCGYPPFYSNHGLAISPGMKTRIRMGQ**<br>**YEFPNPEWSEVSEEVKMLIRNLLKTEPTQRMTITEFMNHPWIMQSTKV**<br>**PQTPLHTSRVLKEDKERWEDVKEEMTSALATMRVDYEQIKIKKIEDAS**<br>**NPLLLKRRKKARALRAAALGH**MEQKLISEEDLK |

**Native sequence**

Amino acids F46 – H400 (end) of human MAPKAP-K2.
Residue F243 of the fusion protein is equilivalent to F46 of the native enzyme. The GST tag is located at residues 1 – 220 and the MYC tag is located at residues 599 – 608. The following amino acid is present after the MAPKAP-K2 sequence and before the MYC tag, M at residue 598 and the following amino acid is present after the MYC tag, K at residue 609.

The following amino acid substitutions are present:
E – R, where E394 of the native sequence is R591 of the fusion protein
A – G, where A399 of the native sequence is G596 of the fusion protein

The enzyme has a D207A mutation.
Residue D207 is equivalent to A404 of the fusion protein.

**Protease cleavage**

Thrombin (LVPRGS) at residues 221 – 226

**Cloning sites** *Bam*H1 and *Eco*R1 sites of pGEX-4T

**Nucleotide Sequence of insert**

TTCCACGTCAAGTCCGGCCTGCAGATCAAGAAGAACGCCATCATCGATGACTACAAGGTCACCAGC
CAGGTCCTGGGGCTGGGCATCAACGGCAAAGTTTTGCAGATCTTCAACAAGAGGACCCAGGAGAAA
TTCGCCCTCAAAATGCTTCAGGACTGCCCCAAGGCCCGCAGGGAGGTGGAGCTGCACTGGCGGGCC
TCCCAGTGCCCGCACATCGTACGGATCGTGGATGTGTACGAGAATCTGTACGCAGGGAGGAAGTGC
CTGCTGATTGTCATGGAATGTTTGGACGGTGGAGAACTCTTTAGCCGAATCCAGGATCGAGGAGAC
CAGGCATTCACAGAAAGAGAAGCATCCGAAATCATGAAGAGCATCGGTGAGGCCATCCAGTATCTG
CATTCAATCAACATTGCCCATCGGGATGTCAAGCCTGAGAATCTCTTATACACCTCCAAAAGGCCC
AACGCCATCCTGAAACTCACTGCCTTTGGCTTTGCCAAGGAAACCACCAGCCACAACTCTTTGACC
ACTCCTTGTTATACACCGTACTATGTGGCTCCAGAAGTGCTGGGTCCAGAGAAGTATGACAAGTCC
TGTGACATGTGGTCCCTGGGTGTCATCATGTACATCCTGCTGTGTGGGTATCCCCCCTTCTACTCC
AACCACGGCCTTGCCATCTCTCCGGGCATGAAGACTCGCATCCGAATGGGCCAGTATGAATTTCCC
AACCCAGAATGGTCAGAAGTATCAGAGGAAGTGAAGATGCTCATTCGGAATCTGCTGAAAACAGAG
CCCACCCAGAGAATGACCATCACCGAGTTTATGAACCACCCTTGGATCATGCAATCAACAAAGGTC
CCTCAAACCCCACTGCACACCAGCCGGGTCCTGAAGGAGGACAAGGAGCGGTGGGAGGATGTCAAG
GAGGAGATGACCAGTGCCTTGGCCACAATGCGCGTTGACTACGAGCAGATCAAGATAAAAAAGATT
GAAGATGCATCCAACCCTCTGCTGCTGAAGAGGCGGAAGAAAGCTCGGGCCCTGGAGGCTGCGGCT
CTGGGCCACATGGAGCAGAAGCTGATCAGCGAGGAGGACCTGAAGtgagtcgac