*Division of Signal Transduction Therapy*

# Standard Operating Procedure

# Preparation of active SAPK3 [1 - 367]

**Enzyme description:-** SAPK3 [1 - 367]

**Clone number:-** DU 980

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Expression level:-** 10 mg/L

**Calculated molecular mass:-** 68, 561 daltons

**Purity:-** >85 %

**Activation protocol:-**
SAPK3 (3.5 µM) is activated in 50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 0.1 mM sodium vanadate, 10 mM MgAc, 0.1 mM ATP with 200 nM MBP-MKK6 DD [DU 1662] at 30 °C for 30 min. Following activation, SAPK3 is re-purified by GSH Sepharose chromatography.

**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:-** Standard filter binding assay

**Assay buffer:-**
50 mM Tris-HCl pH 7.5, 0.1% 2-mercaptoethanol, 0.1mM EGTA, 0.1mM sodium vanadate, 10 mM magnesium acetate

**Substrate:-**
Myelin Basic Protein Final concentration: 0.3 mg/ml

**Specific activity range:-** 100 – 200 U/mg

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# Clone Data Sheet

## SAPK3 [1 – 367]

| | |
|---|---|
| **Protein** | SAPK3 [1 - 367] |
| **Clone number** | DU 980 |
| **Species** | Human |
| **Accession number** | Y10487 |
| **Tags** | N-terminal GST |
| **Bacterially expressed protein** | MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSPEF**MSSPPPARSGF YRQEVTKTAWEVRAVYRDLQPVGSGAYGAVCSAVDGRTGAKVAIKKLY RPFQSELFAKRAYRELRLLKHMRHENVIGLLDVFTPDETLDDFTDFYL VMPFMGTDLGKLMKHEKLGEDRIQFLVYQMLKGLRYIHAAGIIHRDLK PGNLAVNEDCELKILDFGLARQADSEMTGYVVTRWYRAPEVILNWMRY TQTVDIWSVGCIMAEMITGKTLFKGSDHLDQLKEIMKVTGTPPAEFVQ RLQSDEAKNYMKGLPELEKKDFASILTNASPLAVNLLEKMLVLDAEQR VTAGEALAHPYFESLHDTEDEPQVQKYDDSFDDVDRTLDEWKRVTYKE VLSFKPPRQLGARVSKETPL** |
| **Native sequence** | Amino acids M1 – L367 (end) of human SAPK3.<br>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** | Thrombin (LVPRGS) residues 221 - 226 |
| **Cloning sites** | *Eco*RI site of pGEX-4T1 |

**Nucleotide sequence of insert**

ATGAGCTCTCCGCCGCCCGCCCGCAGTGGCTTTTACCGCCAGGAGGTG
ACCAAGACGGCCTGGGAGGTGCGCGCCGTGTACCGGGACCTGCAGCCC
GTGGGCTCGGGCGCCTACGGCGCGGTGTGCTCGGCCGTGGACGGCCGC
ACCGGCGCTAAGGTGGCCATCAAGAAGCTGTATCGGCCCTTCCAGTCC
GAGCTGTTCGCCAAGCGCGCCTACCGCGAGCTGCGCCTGCTCAAGCAC
ATGCGCCACGAGAACGTGATCGGGCTGCTGGACGTATTCACTCCTGAT
GAGACCCTGGATGACTTCACGGACTTTTACCTGGTGATGCCGTTCATG
GGCACCGACCTGGGCAAGCTCATGAAACATGAGAAGCTAGGCGAGGAC
CGGATCCAGTTCCTCGTGTACCAGATGCTGAAGGGGCTGAGGTATATC
CACGCTGCCGGCATCATCCACAGAGACCTGAAGCCCGGCAACCTGGCT
GTGAACGAAGACTGTGAGCTGAAGATCCTGGACTTCGGCCTGGCCAGG
CAGGCAGACAGTGAGATGACTGGTTACGTGGTGACCCGGTGGTACCGG
GCTCCCGAGGTCATCTTGAATTGGATGCGCTACACGCAGACGGTGGAC
ATCTGGTCTGTGGGCTGCATCATGGCGGAGATGATCACAGGCAAGACG
CTGTTCAAGGGCAGCGACCACCTGGACCAGCTGAAGGAGATCATGAAG
GTGACGGGGACGCCTCCGGCTGAGTTTGTGCAGCGGCTGCAGAGCGAT
GAGGCCAAGAACTACATGAAGGGCCTCCCCGAATTGGAGAAGAAGGAT
TTTGCCTCTATCCTGACCAATGCAAGCCCTCTGGCTGTGAACCTCCTG
GAGAAGATGCTGGTGCTGGACGCGGAGCAGCGGGTGACGGCAGGCGAG
GCGCTGGCCCATCCCTACTTCGAGTCCCTGCACGACACGGAAGATGAG
CCCCAGGTCCAGAAGTATGATGACTCCTTTGACGACGTTGACCGCACA
CTGGATGAATGGAAGCGTGTTACTTACAAAGAGGTGCTCAGCTTCAAG
CCTCCCCGGCAGCTGGGGGCCAGGGTCTCCAAGGAGACGCCTCTGtga