

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of IRF3 [1 – 427]

Protein description:- IRF3 [1 - 427]

Clone number:- DU 12079

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 74, 974.77 daltons

Average Mass 75, 022.55 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.41

Purity:- > 85 %

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:- Substrate for TBK1

Division of Signal Transduction Therapy

CLONE DATA SHEET

IRF3 [1 - 427]

Protein IRF3 [1 - 427]

Clone number DU 12079

Species Human

Accession number Z56281.1

Tags N-terminal GST

Bacterially expressed protein MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFOGPLGSPGIPGSTRA
AAMGTPKPRILPWLVSQDLGQLEGVAWVNKSRTFRIPWKHGLRQDA
QQEDFGIFQAWAEATGAYVPGRDKPDLPWKRNFERSALNRKEGLRLAE
DRSKDPHDPHKIYEFVNSGVGDFSQPDTSPTNGGGSTSDTQEDILDE
LLGNMVLAPLPDPGPPSLAVAPEPCQPLRSPSLDNPTFPNLGPISEN
PLKRLVPGEEWFEVTAIFYRGRQVFQQTISCPEGLRLVGSEVGDRTL
PGWPVTLDPGMSLTDGRGMSYVRHVLSCLGGLALWRAGQWLWAQRL
GHCHTYWAVSEELLPNSGHGPDGEVVPKDKEGGVFDLGPFIVDLITFTE
GSGRSPRYALWFCVGESWPQDQPWTKRLVMVKVPTCLRALVEMARVG
GASSENTVDLHISNSHPLSLTSDQYKAYLQDLVEGMDFQGPGES

Native sequence Amino acids M1 – S427 of human IRF3.
Residue M243 of the fusion protein is equivalent to M1 of the native protein. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Division of Signal Transduction Therapy

Cloning sites

NotI sites of pGEX-6P-2

**Nucleotide
sequence of insert**

gcggccgcgATGGGAACCCCAAAGCCACGGATCCTGCCCTGGCTGGTG
TCGCAGCTGGACCTGGGGCAACTGGAGGGCGTGGCCTGGGTGAACAAG
AGCCGCACGCGCTTCCGCATCCCTTGGAAGCACGGCCTACGGCAGGAT
GCACAGCAGGAGGATTCGGAATCTTCCAGGCCTGGGCCGAGGCCACT
GGTGCATATGTTCCCGGAGGGATAAGCCAGACCTGCCAACCTGGAAG
AGGAATTTCCGCTCTGCCCTCAACCGCAAAGAAGGGTTGCGTTTAGCA
GAGGACCGGAGCAAGGACCCTCACGACCCACATAAAATCTACGAGTTT
GTGAACTCAGGAGTTGGGGACTTTTCCAGCCAGACACCTCTCCGGAC
ACCAATGGTGGAGGCAGTACTTCTGATACCCAGGAAGACATTCTGGAT
GAGTTACTGGGTAACATGGTGTGGCCCCACTCCCAGATCCGGGACCC
CCAAGCCTGGCTGTAGCCCCTGAGCCCTGCCCTCAGCCCCTGCGGAGC
CCCAGCTTGGACAATCCCACTCCCTTCCCAAACCTGGGGCCCTCTGAG
AACCCACTGAAGCGGCTGTTGGTGCCGGGGGAAGAGTGGGAGTTCGAG
GTGACAGCCTTCTACCGGGGCCCAAGTCTTCCAGCAGACCATCTCC
TGCCCCGAGGGCCTGCGGCTGGTGGGGTCCGAAGTGGGAGACAGGACG
CTGCCTGGATGGCCAGTCACACTGCCAGACCCTGGCATGTCCCTGACA
GACAGGGGAGTGATGAGCTACGTGAGGCATGTGCTGAGCTGCCTGGGT
GGGGACTGGCTCTCTGGCGGGCCGGGCAGTGGCTCTGGGCCAGCGG
CTGGGGCACTGCCACACATACTGGGCAGTGAGCGAGGAGCTGCTCCCC
AACAGCGGGCATGGGCCTGATGGCGAGGTCCCCAAGGACAAGGAAGGA
GGCGTGTTGACCTGGGGCCCTTCATTGTAGATCTGATTACCTTCACG
GAAGGAAGCGGACGCTCACCACGCTATGCCCTCTGGTTCTGTGTGGGG
GAGTCATGGCCCCAGGACCAGCCGTGGACCAAGAGGCTCGTGATGGTC
AAGGTTGTGCCACGTCCTCAGGGCCTTGGTAGAAATGGCCCGGGTA
GGGGTGCCCTCCTCCCTGGAGAATACTGTGGACCTGCACATTTCCAAC
AGCCACCCACTCTCCCTCACCTCCGACCAGTACAAGGCCTACCTGCAG
GACTTGGTGGAGGGCATGGATTTCCAGGGCCCTGGGGAGAGCtgagcgc
gccgc