

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of active JNK2 alpha 2 [1 - 424]**

|                                           |                                      |
|-------------------------------------------|--------------------------------------|
| <b><u>Enzyme description:-</u></b>        | JNK2 alpha 2 [1 - 424]               |
| <b><u>Clone number:-</u></b>              | DU 699                               |
| <b><u>Source:-</u></b>                    | Recombinant                          |
| <b><u>Expression system:-</u></b>         | Baculovirus expression vector system |
| <b><u>Tag:-</u></b>                       | N-terminal His(6)                    |
| <b><u>Purification method:-</u></b>       | Ni <sup>2+</sup> -NTA agarose        |
| <b><u>Expression level:-</u></b>          | 3-5 mg/L                             |
| <b><u>Calculated molecular mass:-</u></b> | 49, 061 daltons                      |
| <b><u>Purity:-</u></b>                    | >90 %                                |

#### **Activation protocol:-**

JNK2 alpha 2 (2 µM) is activated in 50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.1 mM sodium vanadate, 10 mM MgAc, 0.1 mM ATP with 200 nM activated GST-MKK4 [DU 1788] and 200 nM activated GST-MKK7 beta [DU 703] at 30 °C for 40 min. Following activation, JNK2 is repurified by Ni<sup>2+</sup>-NTA agarose 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, 0.1 mM EGTA, 10 mM MgAc

#### **Substrate:-**

GST-ATF2 [19 - 96] [DU 1787]      Final concentration: 0.2 mg/ml

**Specific activity range:-** 60 - 120 U/mg

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## **Clone Data Sheet**

### **JNK2 alpha 2 [1 – 424]**

|                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|---------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b><u>Protein</u></b>                       | JNK2 alpha 2 [1 – 424]                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b><u>Clone number</u></b>                  | DU 699                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b><u>Species</u></b>                       | Human                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b><u>Accession number</u></b>              | L31951                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b><u>Tags</u></b>                          | N-terminal His(6)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b><u>Baculovirus expressed protein</u></b> | MHHHHHMSDSKCD SQFY SVQVADSTFTVLKRYQQLKPI<br>GSGAQQGIVCAAFDTV LGIN VAVKKLSRPFQ NQTHAKRAY<br>REL VLLKCVNHKNIISLLNVFTPQKTLEEFQDVYLV MEL<br>MDANLCQVIHME LDHERMSYLLYQMLCGIKHLHSAGIIH<br>RDLKPSNI VVKSDCTLKILDFGLARTACTNFM MTPYVVT<br>RYYRAPEVILG MGYKENVDIWSVGCIMGELVKGC VIFQG<br>TDHIDQWNKVIEQLGTPSAEFMKKLQPTVRNYVENRPKY<br>PGIKFEELFPDWIFPSESERDKIKTSQARDLLSKMLVID<br>PDKRISVDEALRHPYITVWYDPAEAEAPPPQIYDAQLEE<br>REHAIEEWKELIYKEVMDWEERSKNGVVKDQPSDAAVSS<br>NATPSQSSSINDISSMSTEQTLASDTDSSLDASTGPLEG<br>CR |
| <b><u>Native sequence</u></b>               | Amino acids M1 – R424 (end) of human JNK2 alpha 2.<br>Residue M8 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 2 - 7.                                                                                                                                                                                                                                                                                                                           |
| <b><u>Protease cleavage</u></b>             | None                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b><u>Cloning sites</u></b>                 | <i>Nde</i> 1 and <i>Xho</i> 1 sites of modified pFastBAC1                                                                                                                                                                                                                                                                                                                                                                                                                                               |

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### Complete nucleotide sequence

ATGCACCATCACCATCACCATATGAGCGACAGTAAATGT  
GACAGTCAGTTTTATAGTGTGCAAGTGGCAGACTCAACC  
TTCAGTGTCTAAAACGTTACCAGCAGCTGAAACCAATT  
GGCTCTGGGGCCCAAGGGATTGTTTGTGCTGCATTTGAT  
ACAGTTCTTGGGATAAATGTTGCAGTCAAGAACTAAGC  
CGTCCTTTTCAGAACCAAACTCATGCAAAGAGAGCTTAT  
CGTGAAC TTGTCCTCTTAAAATGTGTCAATCATAAAAAT  
ATAATTAGTTTGTAAATGTGTTTACACCACAAAAAACT  
CTAGAAGAATTTCAAGATGTGTATTTGGTTATGGAATTA  
ATGGATGCTAACTTATGTCAGGTTATTCACATGGAGCTG  
GATCATGAAAGAATGTCCTACCTTCTTTACCAGATGCTT  
TGTGGTATTAACATCTGCATTCAGCTGGTATAATTCAT  
AGAGATTTGAAGCCTAGCAACATTGTTGTGAAATCAGAC  
TGCACCCTGAAGATCCTTGACTTTGGCCTGGCCCGGACA  
GCGTGCACTAACTTCATGATGACCCCTTACGTGGTGACA  
CGGTACTACCGGGCGCCCGAAGTCATCCTGGGTATGGGC  
TACAAAGAGAACGTTGATATCTGGTCAGTGGGTTGCATC  
ATGGGAGAGCTGGTGAAAGGTTGTGTGATATTCCAAGGC  
ACTGACCATATTGATCAGTGAATAAAGTTATTGAGCAG  
CTGGGAACACCATCAGCAGAGTTCATGAAGAACTTCAG  
CCAACTGTGAGGAATTATGTCGAAAACAGACCAAAGTAT  
CCTGGAATCAAATTTGAAGAACTCTTTCCAGATTGGATA  
TTCCCATCAGAATCTGAGCGAGACAAAATAAAAAACAAGT  
CAAGCCAGAGATCTGTTATCAAAAATGTTAGTGATTGAT  
CCTGACAAGCGGATCTCTGTAGACGAAGCTCTGCGTCAC  
CCATACATCACTGTTTGGTATGACCCCGCCGAAGCAGAA  
GCCCCACCACCTCAAATTTATGATGCCCAGTTGGAAGAA  
AGAGAACATGCAATTGAAGAATGGAAAGAGCTAATTTAC  
AAAGAAGTCATGGATTGGGAAGAAAGAAGCAAGAATGGT  
GTTGTAAAAGATCAGCCTTCAGATGCAGCAGTAAGTAGC  
AACGCCACTCCTTCTCAGTCTTCATCGATCAATGACATT  
TCATCCATGTCCACTGAGCAGACGCTGGCCTCAGACACA  
GACAGCAGTCTTGATGCCTCGACGGGACCCCTTGAAGGC  
TGTCGAtga