

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of ATP Citrate Lyase [1 - 1101]**

**Enzyme description:-** ATP Citrate Lyase [1 - 1101]

**Clone number:-** DU 2461

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 151, 866.59 daltons

Average Mass 151, 964.13 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 6.49

**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**

**ATP Citrate Lyase [1 - 1101]**

**Protein** ATP Citrate Lyase [1 - 1101]

**Clone number** DU 2461

**Species** Human

**Accession number** NM\_001096.2

**Tags** N-terminal GST

**Bacterially expressed protein** MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG  
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA  
VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH  
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS  
KYIAWPLQGWQATFGGGDHPPKSDLEVLFOGPLGSPGIPGSREATSCWG  
APDFAGFVGPVEEAPRTDFGRGRAGLSAAMSAKAISEQTGKELLYKFC  
**TTSAIQNRFKYARVTPDTDWARLLQDHPWLLSQNLVVKPDQLIKRRGKL**  
**GLVGVNLTLDGVKSWLKPRLGQ EATVGKATGFLKNFLIEPFVPHSQAE**  
**FYVCIYATREGDYVLFHHEGGVDVGDVDAKAQKLLVGVDEKLNPEDIK**  
**HLLVHAPEDKKEILASFISGLFNFYEDLYFTYLEINPLVVTKDG VYVLD**  
**LAAKVDATADYICKVKWGDIEFPFPGREAYPEEAYIADLDAKSGASLK**  
**LTLNPKGRIWTMVAGGGASVVYSDTICDLGGVNELANYGEYSGAPSEQ**  
**QTYDYAKTILSLMTREKHPDGKILIIIGGSIANFTNVAATFKGIVRAIRD**  
**YQGPLKEHEVTIFVRRGGPNYQ EGLRVMGEVGGKTTGIPIHVFGTETHMT**  
**AIVGMALGHRPIPNQPPTAAHTANFLLNASGSTSTPAPSRTASFSESRA**  
**DEVAPAKKAKPAMPQDSVSPRSLOGKSTTLFSRHTKAI VWGMQTRAVQ**  
**GMLDFDYVCSRDEPSVAAMVYPFTGDHKQKFYWG HKEILIPVFKNMADA**  
**MRKHPEVDVLINFASLRSAYDSTMETMNYAQIR TIAIIAEGIPEALTRK**  
**LIKKADQKGVTIIGPATVGGIKPGCFKIGNTGGMLDNILASKLYRPGSV**  
**AYVSRSGGMSNELNNIISR TTDGVYEGVAIGGDRYPGSTFMDHVLRYQD**  
**TPGVKMIIVLGEIGGTEEYKICRGIKEGR LTKPIVCWCIGTCATMFSSE**  
**VQFGHAGACANQASETAVAKNQALKEAGVFVPRSFDELGEIIQSVYEDL**  
**VANGVIVPAQEVPPPTVPMDISWARELGLIRKPASFMTSICDERGOELI**  
**YAGMPITEVFKEEMGIGGVLGLLWFQKRLPKYSCQFIEMCLMVTADHGP**  
**AVSGAHNTIICARAGKDLVSSLTSGLLTIGDRFGGALDAAKMF SKAFD**  
**SGIIPMEFVNKMKKEGKLIMGIGHRVKSINNPDMRVQILKDYVRQH FPA**  
**TPLLDYALEVEKITTSKKPNLILNVDGLIGVAFV DMLRNCGSFTREEAD**  
**EYIDIGALNGIFVLGRSMGFI GHYLDQKRLKQGLYRHPWDDISYVLP EPH**  
**MSM**

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- Native sequence**      Amino acids M1 – M1101 (end) of human ATP Citrate Lyase.  
Residue M275 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
- Protease cleavage**      PreScission (LEVLFQGP) residues 221 - 228
- Cloning sites**          *Bam*H1 and *Not*1 of pGEX6P-1

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### **Nucleotide Sequence of Insert:**

ggatccccgggaagctaccagctgctggggtgctccggatthttgcgggggttcgctcgggcctgtggaa  
gaagcgcgcgcacggacttcggcagaggttagagcaggtctctctcgcagccATGTCGGCCAAGGCA  
ATTTTCAGAGCAGACGGGCAAAGAACTCCTTTACAAGTTCATCTGTACCACCTCAGCCATCCAGAAT  
CGGTTCAAGTATGCTCGGGTCACTCCTGACACAGACTGGGCCCGCTTGCTGCAGGACCACCCCTGG  
CTGCTCAGCCAGAACTTGGTAGTCAAGCCAGACCAGCTGATCAAACGTCGTGGAAAACCTTGGTCTC  
GTTGGGGTCAACCTCACTCTGGATGGGGTCAAGTCTGGCTGAAGCCACGGCTGGGACAGGAAGCC  
ACAGTTGGCAAGGCCACAGGCTTCCTCAAGAACTTTCTGATCGAGCCCTTCGTCCCCACAGTCAG  
GCTGAGGAGTTCTATGTCTGCATCTATGCCACCCGAGAAGGGGACTACGTCTGTCCACCACGAG  
GGGGGTGTGGACGTGGGTGATGTGGACGCCAAGGCCCAGAAGCTGCTTGTGGCGTGGATGAGAAA  
CTGAATCCTGAGGACATCAAAAAACACCTGTTGGTCCACGCCCTGAAGACAAGAAAAGAAATCTG  
GCCAGTTTTATCTCCGGCCTCTTCAATTTCTACGAGGACTTGTACTTCACCTACCTCGAGATCAAT  
CCCCTTGTAGTGACCAAAGATGGAGTCTATGTCCTTGACTTGGCGGCCAAGGTGGACGCCACTGCC  
GACTACATCTGCAAAGTGAAGTGGGGTGACATCGAGTTCCTCCCCCTTCGGGCGGGAGGCATAT  
CCAGAGGAAGCCTACATTCAGACCTCGATGCCAAAAGTGGGGCAAGCCTGAAGCTGACCTTGCTG  
AACCCCAAAGGGAGGATCTGGACCATGGTGGCCGGGGTGGCGCCTCTGTCTGTACAGCGATAACC  
ATCTGTGATCTAGGGGTGTCAACGAGCTGGCAAACCTATGGGGAGTACTCAGGCGCCCCCAGCGAG  
CAGCAGACCTATGACTATGCCAAGACTATCCTCTCCCTCATGACCCGAGAGAAGCACCCAGATGGC  
AAGATCCTCATCATTGGAGGCAGCATCGAACTTCACCAACGTGGCTGCCACGTTCAAGGGCATC  
GTGAGAGCAATTCGAGATTACCAGGGCCCCCTGAAGGAGCACGAAGTCACAATCTTTGTCCGAAGA  
GGTGGCCCCAACTATCAGGAGGGCTTACGGGTGATGGGAGAAGTCGGGAAGACCACTGGGATCCCC  
ATCCATGTCTTTGGCACAGAGACTCACATGACGGCCATTGTGGGCATGGCCCTGGGCCACCGGCC  
ATCCCCAACCCAGCCACCCACAGCGGCCACACTGCAAACCTTCCTCCTCAACGCCAGCGGGAGCACA  
TCGACGCCAGCCCCCAGCAGGACAGCATCTTTTTCTGAGTCCAGGGCCGATGAGGTGGCGCTGCA  
AAGAAGGCCAAGCCTGCCATGCCACAAGATTAGTCCCAAGTCCAAGATCCCTGCAAGGAAAGAGC  
ACCACCTCTTCAGCCGCCACACCAAGGCCATTGTGTGGGGCATGCAGACCCGGGCCGTGCAAGGC  
ATGCTGGACTTTGACTATGTCTGCTCCCGAGACGAGCCCTCAGTGGCTGCCATGGTCTACCCTTTC  
ACTGGGGACCACAAGCAGAAGTTTTACTGGGGGCACAAAGAGATCCTGATCCCTGTCTTCAAGAAC  
ATGGCTGATGCCATGAGGAAGCATCCGGAGGTAGATGTGCTCATCAACTTTGCCTCTCTCCGCTCT  
GCCTATGACAGCACCATGGAGACCATGAACTATGCCCAGATCCGGACCATCGCCATCATAGCTGAA  
GGCATCCCTGAGGCCCTCACGAGAAAGCTGATCAAGAAGGCGGACCAGAAGGGAGTGACCATCATC  
GGACCTGCCACTGTTGGAGGCATCAAGCCTGGGTGCTTTAAGATTGGCAACACAGGTGGGATGCTG  
GACAACATCCTGGCCTCCAACTGTACCGCCCAGGCAGCGTGGCCTATGTCTCACGTTCCGGAGGC  
ATGTCCAACGAGCTCAACAATATCATCTCTCGGACCACGGATGGCGTCTATGAGGGCGTGGCCATT  
GGTGGGGACAGGTACCCGGGCTCCACATTCATGGATCATGTGTTACGCTATCAGGACACTCCAGGA  
GTCAAAATGATTGTGGTTCTTGGAGAGATTGGGGGCACTGAGGAATATAAGATTTGCCGGGGCATC  
AAGGAGGGCCGCCTCACTAAGCCCATCGTCTGCTGGTGCATCGGGACGTGTGCCACCATGTTCTCC  
TCTGAGGTCCAGTTTGGCCATGCTGGAGCTTGTGCCAACCAGGCTTCTGAAACTGCAGTAGCCAAG  
AACCAGGCTTTGAAGGAAGCAGGAGTGTGTTGTGCCCGGAGCTTTGATGAGCTTGGAGAGATCATC  
CAGTCTGTATAACGAAGATCTCGTGGCCAATGGAGTCATTGTACCTGCCCAGGAGGTGCCGCCCCCA  
ACCGTGCCCATGGACTACTCCTGGGCCAGGGAGCTTGGTTTGGATCCGCAAACCTGCCCTCGTTCATG  
ACCAGCATCTGCGATGAGCGAGGACAGGAGCTCATCTACGCGGGCATGCCATCACTGAGGTCTTC  
AAGGAAGAGATGGGCATTGGCGGGGTCCTCGGCCTCCTCTGGTTCCAGAAAAGGTTGCCTAAGTAC  
TCTTGCCAGTTCATTGAGATGTGTCTGATGGTGCAGCTGATCACGGGCCAGCCGTCTCTGGAGCC  
CACAACACCATCATTTGTGCGCGAGCTGGGAAAGACCTGGTCTCCAGCCTCACCTCGGGGCTGCTC

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ACCATCGGGGATCGGTTTGGGGGTGCCTTGGATGCAGCAGCCAAGATGTTTCAGTAAAGCCTTTGAC  
AGTGGCATTATCCCATGGAGTTTGTGAACAAGATGAAGAAGGAAGGGAAGCTGATCATGGGCATT  
GGTCACCGAGTGAAGTCGATAAACAACCCAGACATGCGAGTGCAGATCCTCAAAGATTACGTCAGG  
CAGCACTTCCCTGCCACTCCTCTGCTCGATTATGCACTGGAAGTAGAGAAGATTACCACCTCGAAG  
AAGCCAAATCTTATCCTGAATGTAGATGGTCTCATCGGAGTCGCATTTGTAGACATGCTTAGAAAC  
TGTGGGTCCTTTACTCGGGAGGAAGCTGATGAATATATTGACATTGGAGCCCTCAATGGCATCTTT  
GTGCTGGGAAGGAGTATGGGGTTCATTGGACACTATCTTGATCAGAAGAGGCTGAAGCAGGGGCTG  
TATCGTCATCCGTGGGATGATATTTTCATATGTTCTTCCGGAACACATGAGCATGtaagcggccgc