

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active ATXN3L [1 – 355]

<u>Enzyme description:-</u>	ATXN3L [1 - 355]
<u>Clone number:-</u>	DU 23070
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose
<u>Calculated molecular mass:-</u>	
Monoisotopic	67, 528.63 daltons
Average Mass	67, 571.62 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.09
<u>Purity:-</u>	80 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT	
<u>Storage temperature:-</u>	-70 °C

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Clone Data Sheet

ATXN3L [1 - 355]

Protein ATXN3L [1 - 355]

Clone number DU 23070

Species Human

Accession number Q9H3M9

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA
VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLVLFQGPLGSMDF**IFHEKQEGFLC**
AQHCLNNLQGEYFSPVELASIAHQLDDEERMMAEGGVTS E EYLAFLO
QPS ENMDDTGFFSIQVISNALKFWGLEI IHFNNPEYQKLGIDP INERSF
ICNYKQHWFTIRKFGKHWFNLSLLAGPELISDTCLANFLARLQQOAYS
VFVVKGDLPDCEADQLLQIISVEEMDTPKLNKGLVKQKEHRVYKTVLE
KVSEESDESGTSDQDEEDFQRALELSRQETNREDEHLRSTIELSMQGS
GNTSQDLPKTSCVTPASEQPKKIKEDYFEKHQQEQKQQQQSDLP GHSS
YLHERPTTSSRAIESDLSDDISEGTVQAAVD T ILEIMRKNLKI KGEK

Native sequence Amino acids M1 – K355 (end) of human ATXN3L.
Residue M232 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 - 229

Cloning sites *Bam*H1 and *Not*1 sites of pGEX 6P-1

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Nucleotide Sequence

ATGGATTTTCATCTTTCATGAGAAACAGGAAGGTTTCCTGTGTGCTCAGC
ACTGTCTGAACAATCTATTGCAAGGAGAATATTTTAGCCCTGTGGAATT
AGCCTCAATTGCACATCAGCTAGATGAAGAAGAGAGGATGAGAATGGCA
GAAGGAGGAGTCACCAGTGAAGAGTATCTTGCATTTTTTACAGCAGCCTT
CAGAAAACATGGATGATACCGGTTTCTTCTCCATTCAGGTAATAAGCAA
TGCCTTGAAGTTCTGGGGTTTAGAGATCATCCATTTCAATAATCCTGAA
TATCAGAAGCTCGGCATTGATCCTATAAATGAAAAGATCTTTTATATGTA
ATTATAAACAACTGGTTTACTATTAGAAAATTTGGAAAACACTGGTT
TAACTTGAATTCTCTCTTGGCGGGTCCAGAATTAATATCAGATACATGC
CTTGCAAATTTCTTGGCTCGATTACAACAACAAGCATATTCTGTATTTG
TTGTCAAGGGTGATCTGCCAGACTGTGAAGCTGACCAACTCCTGCAGAT
CATCAGTGTCTGAAGAGATGGATACACCAAACCTTAATGGAAAAAATTA
GTAAAACAAAAAGAGCATAGAGTCTATAAACAGTCCTTGAAAAAGTAT
CAGAAGAAAGTGATGAGTCTGGAACATCAGACCAAGATGAGGAGGATTT
TCAGAGGGCCCTTGAACCTAAGCCGCCAAGAAACCAATAGAGAAGATGAA
CATCTCCGCAGTACTATTGAGTTAAGCATGCAAGGTAGTTCCGAAACA
CATCGCAAGATCTTCCAAGACATCATGTGTAACCTCTGCTTCAGAACA
GCCGAAGAAAATAAAAGAAGACTATTTTGAAAAGCATCAGCAGGAACAG
AAGCAGCAGCAACAACAGTCAGATCTGCCGGGCCACAGTTCATACCTAC
ACGAAAGGCCAACAACAAGTTTCGAGAGCAATTGAGAGTGATCTCAGTGA
TGACATCAGTGAAGGCACAGTACAGGCCGCTGTCTGACACCATTTTAGAA
ATTATGAGAAAGAATTTGAAAATCAAAGGGGAAAAAataa