Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-ATXN3

Enzyme description:- GST-ATXN3

Clone number:- DU35448

Source:- BL21

Tag:- N-terminal GST tag

Purification method:- Glutathione sepharose

Expression level:- 1.5 mg/L

Calculated molecular mass:-

Monoisotopic 69183 Da
Average Mass 69225 Da
[cycsteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.86

Purity:- 90%

Enzyme storage buffer:-

50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT

Storage temperature:- -80ºC

Assay:-

Ub-Rho110-Gly cleavage assay monitored by Ex/Em 485/535 nm

Assay buffer:-

40 mM Tris pH 7.5, 100 mM NaCl, 5 mM DTT, 0.01% Triton X-100, 0.005% Ovalbumin, 0.5 μM Ub-Rho110-Gly

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Clone Data Sheet

GST-ATXN3

Protein	GST-ATXN3
Synonyms	ATX3, MJD, MJD1, SCA3
Clone Number	DU35448
Species	Human
Accession Number	DNA: AAH33711.1
Tags	N-terminal GST tag
Amino acid sequence of expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIA WPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MESIFHEKQEGSLCAQHCL NNLLQGEYFSPVELSSIAHQLDEEERMRMAEGGVTSEDYRTFLQQPSGNM DDSGFFSIQVISNALKVWGLELILFNSPEYQRLRIDPINERSFICNYKEH WFTVRKLGKQWFNLNSLLTGPELISDTYLALFLAQLQQEGYSIFVVKGDL PDCEADQLLQMIRVQQMHRPKLIGEELAQLKEQRVHKTDLERVLEANDGS GMLDEDEEDLQRALALSRQEIDMEDEEADLRRAIQLSMQGSSRNISQDMT QTSGTNLTSEELRKRREAYFEKQQQKQQQQQQQQQQQQQQQQQQQQGDLSG QSSHPCERPATSSGALGSDLGDAMSEEDMLQAAVTMSLETVRNDLKTEGK K**
Native sequence	in bold This is not the reference sequence for human ATXN3! This sequence is different at the c-terminus, but it agrees with a particular cDNA (AAH33711.1)
Protease cleavage	Precission site underlined
Cloning sites	BamH1 / Not1

DNA sequence of insert

GGATCCATGGAGTCCATCTTCCACGAGAAACAAGAAGGCTCACTTTGTGC
TCAACATTGCCTGAATAACTTATTGCAAGGAGAATATTTTAGCCCTGTGG
AATTATCCTCAATTGCACATCAGCTGGATGAGGAGGAGAGGATGAGAATG
GCAGAAGGAGGAGTTACTAGTGAAGATTATCGCACGTTTTTACAGCAGCC
TTCTGGAAATATGGATGACAGTGGTTTTTTCTCTATTCAGGTTATAAGCA
ATGCCTTGAAAGTTTGGGGTTTAGAACTAATCCTGTTCAACAGTCCAGAG
TATCAGAGGCTCAGGATCGATCCTATAAATGAAAGATCATTTATATGCAA
TTATAAGGAACACTGGTTTACAGTTAGAAAATTAGGAAAACAGTGGTTTA
ACTTGAATTCTCTCTTGACGGGTCCAGAATTAATATCAGATACATATCTT
GCACTTTTCTTGGCTCAATTACAACAGGAAGGTTATTCTATATTTGTCGT
TAAGGGTGATCTGCCAGATTGCGAAGCTGACCAACTCCTGCAGATGATTA
GGGTCCAACAGATGCATCGACCAAAACTTATTGGAGAAGAATTAGCACAA
CTAAAAGAGCAAAGAGTCCATAAAACAGACCTGGAACGAGTGTTAGAAGC
AAATGATGGCTCAGGAATGTTAGACGAAGATGAGGAGGATTTGCAGAGGG
CTCTGGCACTAAGTCGCCAAGAAATTGACATGGAAGATGAGGAAGCAGAT
CTCCGCAGGGCTATTCAGCTAAGTATGCAAGGTAGTTCCAGAAACATATC
TCAAGATATGACACAGACATCAGGTACAAATCTTACTTCAGAAGAGCTTC
GGAAGAGACGAGAAGCCTACTTTGAAAAACAGCAGCAAAAGCAGCAACAG
CAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGGG
GGACCTATCAGGACAGAGTTCACATCCATGTGAAAGGCCAGCCACCAGTT
CAGGAGCACTTGGGAGTGATCTAGGTGATGCTATGAGTGAAGAAGACATG
CTTCAGGCAGCTGTGACCATGTCTTTAGAAACTGTCAGAAATGATTTGAA
AACAGAAGGAAAAAAATAAGCGGCCGC