

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Forkhead box protein O3 [2 – 673]

<u>Enzyme description:-</u>	FOXO3A [2 – 673]
<u>Clone number:-</u>	DU 1716
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E. coli</i>
<u>Tag:-</u>	N-terminal GST and C-terminal His6
<u>Purification method:-</u>	GSH Sepharose
<u>Calculated molecular mass:-</u>	
Monoisotopic	98,730.12 daltons
Average Mass	98,792.45 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.39
<u>Purity:-</u>	80 %
<u>Enzyme storage buffer:-</u>	
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine	
<u>Storage temperature:-</u>	-70 °C

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Clone Data Sheet

FOXO3A [2 – 673]

Protein FOXO3A [2 – 673]

Clone number DU 1716

Species Human

Accession number NP_001446

Tags N-terminal GST

Bacterially expressed protein MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKK
FELGLEFPNLPYYIDGDVKLQSMAIIRYIADKHNMLGGCPKERA
EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFED
RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVL
QGPLGSAEAPASPAPLSPLEVELDPEFEPQSRPRSCWTPLQRP
ELQASPAKPSGETAADSMIPEEEDDEDDEDGGGRAGSAMAIGGGGS
GTLGSGLLLED SARVLAPGGQDPGSGPATAAGGLSGGTQALLQP
QPLPPPQPGAAGSGQPRKCSSRRNAWGNLSYADLITRAIESSPD
KRLTLSQIYEMVRCVPYFKDKGDSNSSAGWKN SIRHNSLHSRF
MRVQNEGTGKSSWI INPDGGKSGKAPRRRAVSMDNSNKYTKSRG
RAAKKKAALQTAPESADDSPSQLSKWPGSPTSRSSELDATWDFR
SRTNSNASTVSGRLSPIMASTELDEVQDDAPLSPMLYSSASLS
PSVSKPCTVELPRLTDMAGTMNLNDGLTENLMDDLLDNITLPPSQ
PSPTGGLMQRSSSFYTTKGSGLSPTSSFNSTVFGPSSLNSLRQ
SPMQTIQENKPATFSSMSHYGNQTLQDLLTSDSLSHSDVMMTQSD
PLMSQASTAVSAQNSRRNVMLRNDPMMSFAAQPNOGSLVNQNLH
HQHQTQALGGSRALSNSVSNMGLSESSSLGSAKHQQSPVSQSM
QTLSDSLSGSSLYSTSANLPVMGHEKFPDDLDMFNGSLECDME
SIIRSELMDADGLDFNFDSLITQNVVGLNVGNFTGAKQASSQSW
VPGHHHHHH

Native sequence Amino acids A2 – G673 (end) of human.
Residue A232 of the fusion protein is equivalent to A2 of the native enzyme. The GST tag is located at residues 1 – 220 and the His6 tag is located at residues 904 – 909.

Protease cleavage PreScission (LEVLFQGP) residues 220 - 227

Cloning sites *Bam*H1 and *Sal*I sites of pGex6P-1

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Nucleotide insert

ggatccGCAGAGGCACCGGCTTCCCCGGCCCCGCTCTCTCCGCTC
GAAGTGGAGCTGGACCCGGAGTTCGAGCCCCAGAGCCGTCCGCGA
TCCTGTACGTGGCCCCTGCAAAGGCCGGAGCTCCAAGCGAGCCCT
GCCAAGCCCTCGGGGGAGACGGCCGCTGACTCCATGATCCCCGAG
GAGGAGGACGATGAAGACGACGAGGACGGCGGGGGACGGGCCGGC
TCGGCCATGGCGATCGGCGGGCGGCGGGAGCGGCACGCTGGGC
TCCGGGCTGCTCCTTGAGGACTCGGCCCGGGTGCTGGCACC CGA
GGCAAGACCCCGGTCTGGGCCAGCCACCGCGGCGGGCGGGCTG
AGCGGGGTACACAGGCGTGTGCAGCCTCAGCAACCGCTGCCA
CCGCCGACGCCGGGGCGGCTGGGGGCTCCGGGCAGCCGAGGAAA
TGTTTCGTCGCGGCGGAACGCCTGGGGAAACCTGTCTTACGCGGAC
CTGATCACCCGCGCCATCGAGAGCTCCCCGGACAAACGGCTCACT
CTGTCCCAGATCTACGAGTGGATGGTTCGTTGCGTGCCCTACTTC
AAGGATAAGGGCGACAGCAACAGCTCTGCCGGCTGGAAGAACTCC
ATCCGGCACAACCTGTCACTGCATAGTCGATTCATGCGGGTCCAG
AATGAGGGAACCTGGCAAGAGCTCTTGGTGGATCATCAACCCTGAT
GGGGGGAAGAGCGGAAAAGCCCCCGGCGGCGGGCTGTCTCCATG
GACAATAGCAACAAGTATAACCAAGAGCCGTGGCCGCGCAGCCAAG
AAGAAGGCAGCCCTGCAGACAGCCCCGAATCAGCTGACGACAGT
CCCTCCCAGCTCTCCAAGTGGCCTGGCAGCCCCACGTCACGCAGC
AGTGATGAGCTGGATGCGTGGACGGACTTCCGTTACGCACCAAT
TCTAACGCCAGCACAGTCAGTGGCCGCTGTGCGCCATCATGGCA
AGCACAGAGTTGGATGAAGTCCAGGACGATGATGCGCCTCTCTCG
CCCATGCTCTACAGCAGCTCAGCCAGCCTGTACCTTCAGTAAGC
AAGCCGTGCACGGTGGAACTGCCACGGCTGACTGATATGGCAGGC
ACCATGAATCTGAATGATGGGCTGACTGAAAACCTCATGGACGAC
CTGCTGGATAACATCACGCTCCCGCCATCCAGCCATCGCCCACT
GGGGGACTCATGCAGCGGAGCTCTAGCTTCCCGTATAACCACCAAG
GGCTCGGGCCTGGGCTCCCCAACCCAGCTCCTTTAACAGCACGGTG
TTCGGACCTTCATCTCTGAACTCCCTACGCCAGTCTCCCATGCAG
ACCATCCAAGAGAACAAGCCAGCTACCTTCTCTTCCATGTCACAC
TATGGTAACCAGACACTCCAGGACCTGCTCACTTCGGACTCACTT
AGCCACAGCGATGTCATGATGACACAGTCGGACCCCTTGATGTCT
CAGGCCAGCACCGCTGTGTCTGCCAGAATTCCCGCCGGAACGTG
ATGCTTCGCAATGATCCGATGATGTCCTTTGCTGCCCAGCCTAAC
CAGGGAAGTTTGGTCAATCAGAACTTGCTCCACCACCAGCACCAA
ACCCAGGGCGCTCTTGGTGGCAGCCGTGCCTTGTCGAATTCTGTC
AGCAACATGGGCTTGAGTGAGTCCAGCAGCCTTGGGTCAGCCAAA
CACCAGCAGCAGTCTCCTGTGACCCAGTCTATGCAAACCTCTCG
GACTCTCTCTCAGGCTCCTCCTTGTAAGTCAACTAGTGCAAACCTG
CCCGTCATGGGCCATGAGAAGTTCCCAGCGACTTGGACCTGGAC
ATGTTCAATGGGAGCTTGGAAATGTGACATGGAGTCCATTATCCGT
AGTGAACCTCATGGATGCTGATGGGTTGGATTTTAACTTTGATTCC
CTCATCTCCACACAGAATGTTGTTGGTTTGAACGTGGGGAACTTC
ACTGGTGCTAAGCAGGCTCATCTCAGAGCTGGGTGCCAGGCCAT
CATACCATCACCATtaagtcgacgc