

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Cofilin 2 S3A [1 - 166] (muscle isoform)

<u>Protein description:-</u>	Cofilin 2 S3A [1 - 166] (muscle isoform)
<u>Clone number:-</u>	DU 428
<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>Expression level:-</u>	4 mg/L
<u>Calculated molecular mass:-</u>	45, 515 daltons
<u>Purity:-</u>	95 %
<u>Enzyme storage buffer:-</u>	
	50 mM Tris/HCl pH 7.5, 50 % glycerol, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.
<u>Storage temperature:-</u>	-20 °C
<u>Assay:-</u>	Control substrate for LIMK1

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CLONE DATA SHEET

Cofilin 2 S3A [1 - 166] (muscle isoform)

<u>Protein</u>	Cofilin 2 S3A [1 - 166] (muscle isoform)
<u>Clone number</u>	DU 428
<u>Species</u>	Human
<u>Accession no</u>	NM_021914
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKW RNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNML GGCPKERAIEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFL SKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLY MDPMCLDAFPKLVCFKKRIEAIIPQIDKYLKSSKYIAWPLQG WQATFGGGDHPKSDLEVLFGPLGSMAAGVTVNDEVIKVF NDMKVRKSSTQEEIKKRKKAVLFCLSDDKRQIIVEEAKQIL VGDIGDTVEDPYTSFVKLLPLNDCRYALYDATYETKESKKE DLVFIWAPESAPLKSKMIYASSKDAIKKKFTGIKHEWQVN GLDDIKDRSTLGEKLGGNVVVSLEGKPL</p>
<u>Native sequence</u>	<p>Amino acids M1 – L166 (end) of human Cofilin 2. Residue M232 of the fusion protein is equivalent to M1 of the native protein. The protein has a S3A mutation, where S3 which is phosphorylated by LIMK1 has been changed to A. Residue S3 is equivalent to residue A234 of the fusion protein. The GST tag is located at residues 1 - 220</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGPL</u>) residues 221 - 229
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Sal</i> I of pGEX-6P-1

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Nucleotide
sequence of insert

ATGGCTGCTGGAGTTACAGTGAATGATGAAGTCATCAAAGT
TTTTAATGATATGAAAGTAAGGAAATCTTCTACACAAGAGG
AGATCAAAAAGAGAAAGAAAGCAGTTCTCTTCTGTTTAAGC
GATGACAAAAGACAAATAATTGTAGAGGAAGCAAAGCAGAT
CTTGGTGGGTGACATTGGTGATACTGTAGAGGACCCCTACA
CATCTTTTGTGAAGTTGCTACCTCTGAATGATTGCCGATAT
GCTTTGTACGATGCCACATACGAAACAAAAGAGTCTAAGAA
AGAAGACCTAGTATTTATATTCTGGGCTCCTGAAAGTGCAC
CTTTAAAAAGCAAGATGATTTATGCTAGCTCTAAAGATGCC
ATTAAAAAGAAATTTACAGGTATTAAACATGAGTGGCAAGT
AAATGGCTTGGATGATATTAAGGACCGTTCGACACTTGGAG
AGAAATTGGGAGGCAATGTAGTAGTTTCACTTGAAGGAAAA
CCATTAtaa