

Division of Signal Transduction Therapy

Standard Operating Procedure

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

<u>Protein description:-</u>	Cofilin 2 [1 - 166] (muscle isoform)
<u>Clone number:-</u>	DU 427
<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, 531 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>	Substrate for LIMK1

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CLONE DATA SHEET

Cofilin 2 [1 - 166] (muscle isoform)

<u>Protein</u>	Cofilin 2 [1 - 166] (muscle isoform)
<u>Clone number</u>	DU 427
<u>Species</u>	Human
<u>Accession no</u>	NM_021914
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKW RNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNML GGCPKERAIEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFL SKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLY MDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQG WQATFGGGDHPPKSDLEVLFGGPLGSMASGVTVNDEVIKVF NDMKVRKSSTQEEIKRKKAVLFLCLSDDKRQIIVEEAKQIL 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 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> 1 of pGEX-6P-1

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Nucleotide
sequence of insert

GGATCCATGGCTTCTGGAGTTACAGTGAATGATGAAGTCAT
CAAAGTTTTTAATGATATGAAAGTAAGGAAATCTTCTACAC
AAGAGGAGATCAAAAAGAGAAAGAAAGCAGTTCTTCTGT
TTAAGCGATGACAAAAGACAAATAATTGTAGAGGAAGCAAA
GCAGATCTTGGTGGGTGACATTGGTGATACTGTAGAGGACC
CCTACACATCTTTTGTGAAGTTGCTACCTCTGAATGATTGC
CGATATGCTTTTGTACGATGCCACATACGAAACAAAAGAGTC
TAAGAAAGAAGACCTAGTATTTATATTCTGGGCTCCTGAAA
GTGCACCTTTAAAAAGCAAGATGATTTATGCTAGCTCTAAA
GATGCCATTTAAAAAGAAATTTACAGGTATTAACATGAGTG
GCAAGTAAATGGCTTGGATGATATTAAGGACCGTTCGACAC
TTGGAGAGAAATTGGGAGGCAATGTAGTAGTTTCACTTGAA
GGAAAACCATTAtaagtcgac