Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of NogoB [2 – 373]

Protein description:-	NogoB [2 – 373]
Clone number:-	DU 092
Source:-	Recombinant
Expression system:-	*E.coli*
Tag:-	N-terminal GST and HA
Purification method:-	GSH Sepharose
Expression level:-	1 mg/L
Calculated molecular mass:-	68, 355 daltons
Purity:-	50 %

Enzyme storage buffer:-

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.

Storage temperature:-	–20 °C

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CLONE DATA SHEET

NogoB [2 – 373]

Protein	NogoB [2 – 373]
Clone number	DU 092
Species	Human
Accession no	AJ251384
Tags	N-terminal GST and HA
Bacterially expressed prtoein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRN KKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCP KERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEM LKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDA FPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDH PPKSDLEVLFQGPLGSATMYPYDVPDYA**EDLDQSPLVSSSDSP** **PRPQPAFKYQFVREPEDEEEEEEEEEEDEDEDLEELEVLERKP** **AAGLSAAPVPTAPAAGAPLMDFGNDFVPPAPRGPLPAAPPVAP** **ERQPSWDPSPVSSTVPAPSPLSAAAVSPSKLPEDDEPPARPPP** **PPPASVSPQAEPVWTPPAPAPAAPPSTPAAPKRRGSSGSVVVD** **LLYWRDIKKTGVVFGASLFLLLSLTVFSIVSVTAYIALALLSV** **TISFRIYKGVIQAIQKSDEGHPFRAYLESEVAISEELVQKYSN** **SALGHVNCTIKELRRLFLVDDLVDSLKFAVLMWVFTYVGALFN** **GLTLLILALISLFSVPVIYERHQAQIDHYLGLANKNVKDAMAK** **IQAKIPGLKRKAE**
Native sequence	Amino acids E2 – E373 (end) of human NogoB. Residue E244 of the fusion protein is equivalent to E2 of the native protein. The GST tag is located at residues 1 – 220 and the HA tag [YPYDVPDYA] is located at residues 235 – 243.
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Bam*H1 of pGEX 6P-1

Nucleotide sequence of insert

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ggatccGCCACCATGTACCCATACGATGTGCCAGATTACGCCGAAGACCT
GGACCAGTCTCCTCTGGTCTCGTCCTCGGACAGCCCACCCCGGCCGCAGC
CCGCGTTCAAGTACCAGTTCGTGAGGGAGCCCGAGGACGAGGAGGAAGAA
GAGGAGGAGGAAGAGGAGGACGAGGACGAAGACCTGGAGGAGCTGGAGGT
GCTGGAGAGGAAGCCCGCCGCCGGGCTGTCCGCGGCCCCAGTGCCCACCG
CCCCTGCCGCCGGCGCGCCCCTGATGGACTTCGGAAATGACTTCGTGCCG
CCGGCGCCCCGGGGACCCCTGCCGGCCGCTCCCCCCGTCGCCCCGGAGCG
GCAGCCGTCTTGGGACCCGAGCCCGGTGTCGTCGACCGTGCCCGCGCCAT
CCCCGCTGTCTGCTGCCGCAGTCTCGCCCTCCAAGCTCCCTGAGGACGAC
GAGCCTCCGGCCCGGCCTCCCCCTCCTCCCCCGGCCAGCGTGAGCCCCCA
GGCAGAGCCCGTGTGGACCCCGCCAGCCCCGGCTCCCGCCGCGCCCCCCT
CCACCCCGGCCGCGCCCAAGCGCAGGGGCTCCTCGGGCTCAGTGGTTGTT
GACCTCCTGTACTGGAGAGACATTAAGAAGACTGGAGTGGTGTTTGGTGC
CAGCCTATTCCTGCTGCTTTCATTGACAGTATTCAGCATTGTGAGCGTAA
CAGCCTACATTGCCTTGGCCCTGCTCTCTGTGACCATCAGCTTTAGGATA
TACAAGGGTGTGATCCAAGCTATCCAGAAATCAGATGAAGGCCACCCATT
CAGGGCATATCTGGAATCTGAAGTTGCTATATCTGAGGAGTTGGTTCAGA
AGTACAGTAATTCTGCTCTTGGTCATGTGAACTGCACGATAAAGGAACTC
AGGCGCCTCTTCTTAGTTGATGATTTAGTTGATTCTCTGAAGTTTGCAGT
GTTGATGTGGGTATTTACCTATGTTGGTGCCTTGTTTAATGGTCTGACAC
TACTGATTTTGGCTCTCATTTCACTCTTCAGTGTTCCTGTTATTTATGAA
CGGCATCAGGCACAGATAGATCATTATCTAGGACTTGCAAATAAGAATGT
TAAAGATGCTATGGCTAAAATCCAAGCAAAAATCCCTGGATTGAAGCGCA
AAGCTGAAtga
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