Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of Laforin [2 - 331]

Enzyme description:- Laforin [2 – 331]

Clone number:- DU 1620

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 63, 809.03 daltons
Average Mass 63, 850.53 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.01

Purity:- >80 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Division of Signal Tranduction Therapy

Clone Data Sheet

Laforin [2 – 331]

Protein Laforin [2 – 331]

Clone number DU 1620

Species Human

Accession number NM_005670.3

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**RFRFGVVVPPAVAG**
ARPELLVVGSRPELGRWEPRGAVRLRPAGTAAGDGALALQEPGLWLGEV
ELAAEEAAQDGAEPGRVDTFWYKFLKREPGGELSWEGNGPHHDRCCTYN
ENNLVDGVYCLPIGHWIEATGHTNEMKHTTDFYFNIAGHQAMHYSRILP
NIWLGSCPRQVEHVTIKLKHELGITAVMNFQTEWDIVQNSSGCNRYPEP
MTPDTMIKLYREEGLAYIWMPTPDMSTEGRVQMLPQAVCLLHALLEKGH
IVYVHCNAGVGRSTAAVCGWLQYVMGWNLRKVQYFLMAKRPAVYIDEEA
LARAQEDFFQKFGKVRSSVCSL

Native sequence Amino acids R2 – L331 (end) of human Laforin.
Residue R232 of the fusion protein is equivalent to R2 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Bam*H1 sites of pGEX6P-1

Nucleotide Sequence Of Insert

ggatccCGCTTCCGCTTTGGGGTGGTGGTGCCACCCGCCGTGGCCGGCG
CCCGGCCGGAGCTGCTGGTGGTGGGGTCGCGGCCCGAGCTGGGGCGTTG
GGAGCCGCGCGGTGCCGTCCGCCTGAGGCCGGCCGGCACCGCGGCGGGC
GACGGGGCCCTGGCGCTGCAGGAGCCGGGCCTGTGGCTCGGGGAGGTGG
AGCTGGCGGCCGAGGAGGCGGCGCAGGACGGGGCGGAGCCGGGCCGCGT
GGACACGTTCTGGTACAAGTTCCTGAAGCGGGAGCCGGGAGGAGAGCTC
TCCTGGGAAGGCAATGGACCTCATCATGACCGTTGCTGTACTTACAATG
AAAACAACTTGGTGGATGGTGTGTATTGTCTCCCAATAGGACACTGGAT
TGAGGCCACTGGACACACCAATGAAATGAAGCACACAACAGACTTCTAT
TTTAATATTGCAGGCCACCAAGCCATGCATTATTCAAGAATTCTACCAA
ATATCTGGCTGGGTAGCTGCCCTCGTCAGGTGGAACATGTAACCATCAA
ACTGAAGCATGAATTGGGGATTACAGCTGTAATGAATTTCCAGACTGAA
TGGGATATTGTACAGAATTCCTCAGGCTGTAACCGCTACCCAGAGCCCA
TGACTCCAGACACTATGATTAAACTATATAGGGAAGAAGGCTTGGCCTA
CATCTGGATGCCAACACCAGATATGAGCACCGAAGGCCGAGTACAGATG
CTGCCCCAGGCGGTGTGCCTGCTGCATGCGCTGCTGGAGAAGGGACACA
TCGTGTACGTGCACTGCAACGCTGGGGTGGGCCGCTCCACCGCGGCTGT
CTGCGGCTGGCTCCAGTATGTGATGGGCTGGAATCTGAGGAAGGTGCAG
TATTTCCTCATGGCCAAGAGGCCGGCTGTCTACATTGACGAAGAGGCCT
TGGCCCGGGCACAAGAAGATTTTTTCCAGAAATTTGGGAAGGTTCGTTC
TTCTGTGTGTAGCCTGtagggatcc

Division of Signal Tranduction Therapy