Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of PALMD [1 – 551]

Enzyme description:- PALMD [1 – 551]

Clone number:- DU 48940

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 90, 265.73 daltons
Average Mass 90, 322.01 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.40

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

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Clone Data Sheet

PALMD [1 – 551]

Protein PALMD [1 – 551]

Clone number DU 48940

Species Human

Accession number NM_017734.3

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPNSRVDA**MEEAELV**
KGRLQAITDKRKIQEEISQKRLKIEEDKLKHQHLKKKALREKWLLDGIS
SGKEQEEMKKQNQQDQHQIQVLEQSILRLEKEIQDLEKAELQISTKEEA
ILKKLKSIERTTEDIIRSVKVEREERAEESIEDIYANIPDLPKSYIPSR
LRKEINEEKEDDEQNRKALYAMEIKVEKDLKTGESTVLSSIPLPSDDFK
GTGIKVYDDGQKSVYAVSSNHSAAYNGTDGLAPVEVEELLRQASERNSK
SPTEYHEPVYANPFYRPTTPQRETVTPGPNFQERIKIKTNGLGIGVNES
IHNMGNGLSEERGNNFNHISPIPPVPHPRSVIQQAEEKLHTPQKRLMTP
WEESNVMQDKDAPSPKPRLSPRETIFGKSEHQNSSPTCQEDEEDVRYNI
VHSLPPDINDTEPVTMIFMGYQQAEDSEEDKKFLTGYDGIIHAELVVID
DEEEEDEGEAEKPSYHPIAPHSQVYQPAKPTPLPRKRSEASPHENTNHK
SPHKNSISLKEQEESLGSPVHHSPFDAQTTGDGTEDPSLTALRMRMAKL
GKKVI

Native sequence Amino acids M1 – I551 (end) of human PALMD.
Residue M239 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Sal*1 and *Not*1 sites of pGEX6P-3

Nucleotide Sequence of insert

gtcgacgccATGGAAGAAGCTGAGCTGGTGAAGGGAAGACTCCAGGCCA
TCACAGATAAAAGAAAAATACAGGAAGAAATCTCACAGAAGCGTCTGAA
AATAGAGGAAGACAAACTAAAGCACCAGCATTTGAAGAAAAAGGCCTTG
AGGGAGAAATGGCTTCTAGATGGAATCAGCAGCGGAAAAGAACAGGAAG
AGATGAAGAAGCAAAATCAACAAGACCAGCACCAGATCCAGGTTCTAGA
ACAAAGTATCCTCAGGCTTGAGAAAGAGATCCAAGATCTTGAAAAAGCT
GAACTGCAAATCTCAACGAAGGAAGAGGCCATTTTAAAGAAACTAAAGT
CAATTGAGCGGACAACAGAAGACATTATAAGATCTGTGAAAGTGGAAAG
AGAAGAAAGAGCAGAAGAGTCAATTGAGGACATCTATGCTAATATCCCT
GACCTTCCAAAGTCCTACATACCTTCTAGGTTAAGGAAGGAGATAAATG
AAGAAAAAGAAGATGATGAACAAAATAGGAAAGCTTTATATGCCATGGA
AATTAAAGTTGAAAAAGACTTGAAGACTGGAGAAAGTACAGTTCTGTCT
TCAATACCTCTGCCATCAGATGACTTTAAAGGTACAGGAATAAAAGTTT
ATGATGATGGGCAAAAGTCAGTGTATGCAGTAAGTTCTAATCACAGTGC
AGCATACAATGGCACCGATGGCCTGGCACCAGTTGAAGTAGAGGAACTT
CTAAGACAAGCCTCAGAGAGAAACTCTAAATCCCCAACAGAGTATCATG
AGCCTGTATATGCCAATCCCTTTTACAGGCCTACAACCCCACAGAGAGA
AACGGTGACCCCTGGACCAAACTTTCAAGAAAGGATAAAGATTAAAACT
AATGGACTGGGTATTGGTGTAAATGAATCCATACACAATATGGGCAATG
GTCTTTCAGAGGAAAGGGGAAACAACTTCAATCACATCAGTCCCATTCC
GCCAGTGCCTCATCCCCGATCAGTGATTCAACAAGCAGAAGAGAAGCTT
CACACCCCGCAAAAAAGGCTAATGACTCCTTGGGAAGAATCGAATGTCA
TGCAGGACAAAGATGCACCCTCTCCAAAGCCAAGGCTGAGCCCCAGAGA
GACAATATTTGGGAAATCTGAACACCAGAATTCTTCACCCACTTGTCAG
GAGGACGAGGAAGATGTCAGATATAATATCGTTCATTCCCTGCCTCCAG
ACATAAATGATACAGAACCGGTGACAATGATTTTCATGGGGTATCAGCA
GGCAGAAGACAGTGAAGAAGATAAGAAGTTTCTGACAGGATATGATGGG
ATCATCCATGCTGAGCTGGTTGTGATTGATGATGAGGAGGAGGAGGATG
AAGGAGAAGCAGAGAAACCGTCCTACCACCCCATAGCTCCCCATAGTCA
GGTGTACCAGCCAGCCAAACCAACACCACTTCCTAGAAAAAGATCAGAA
GCTAGTCCTCATGAAAACACAAATCATAAATCCCCCCACAAAAATTCCA
TATCTCTGAAAGAGCAAGAAGAAAGCTTAGGCAGCCCTGTCCACCATTC
CCCATTTGATGCTCAGACAACTGGAGATGGGACTGAGGATCCATCCTTA
ACAGCTTTAAGGATGAGAATGGCAAAGCTGGGAAAAAAGGtgatctaag
cggccgc

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