Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of Green Fluorescent Protein [2 – 238]

Protein description:- Green Fluorescent Protein [2 – 238]

Clone number:- DU 1574

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 10 mg/L

Calculated molecular mass:- 53, 600 daltons

Purity:- > 95 %

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

Green Fluorescent Protein [2 – 238]

Protein	Green Fluorescent Protein [2 – 238]
Clone number	DU 1574
Species	Aequorea victoria
Accession number	M62653
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLG**SVSKGEELFT** **GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPW** **PTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGN** **YKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIM** **ADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYL** **STQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK**
Native sequence	Amino acids S2 – K238 (end) of Aequorea victoria Green Fluorescent Protein. Residue S231 of the fusion protein is equivalent to S2 of the native protein. The GST tag is located at residues 1 – 220. The following amino acid substitutions are present: F – L, where F64 of the native sequence is L295 of the fusion protein. S – T, where S65 of the native sequence is T296 of the fusion protein. H – L, where H231 of the native sequence is L462 of the fusion protein.
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Bam*HI and *Eco*RI sites of pGEX-6P-1

Nucleotide sequence of insert

GGATCCGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATC
CTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCC
GGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTC
ATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACC
ACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATG
AAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAG
GAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCC
GAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAG
GGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAG
TACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAG
AACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGC
AGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGAC
GGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCC
CTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAG
TTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG
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