Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of Ceramide Synthase 5 [1 – 65]

Enzyme description:- Ceramide Synthase 5 [1 – 65]

Clone number:- DU 46828

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 33, 651.15 daltons
Average Mass 33, 673.06 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.85

Purity:- >75 %

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

Ceramide Synthase 5 [1 – 65]

Protein	Ceramide Synthase 5 [1 – 65]
Clone number	DU 46828
Species	Human
Accession number	XM_005269220.1
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLVPRGS**MATAAQGPLSLLWGWLWSE** **RFWLPENVSWADLEGPADGYGYPRGRHILSVFPLAAGIFFVRLLFE**
Native sequence	Amino acids M1 – E65 (end residue M409) of human Ceramide Synthase 5. Residue M227 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	Thrombin (LVPRGS) residues 221 - 226
Cloning sites	*Bgl*II *Not*1 into *Bam*H1 *Not*1 sites of pGEX4T1

Complete Nucleotide Sequence

gatctggttccgcgtggATCGATGGCGACAGCAGCGCAGGGACCCCTAA
GCTTGCTGTGGGGCTGGCTGTGGAGCGAGCGCTTCTGGCTACCCGAGAA
CGTGAGCTGGGCTGATCTGGAGGGGCCGGCCGACGGCTACGGTTACCCC
CGCGGCCGGCACATCCTCTCGGTGTTCCCGCTGGCGGCGGGCATCTTCT
TCGTGAGGCTGCTCTTCGAGtgatcgagcggccgc