Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of NCC [1 - 100]

Enzyme description:- NCC [1 - 100]

Clone number:- DU 4965

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 37, 806.91 daltons
Average Mass 37, 831.35 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.75

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

NCC [1 - 100]

Protein	NCC [1 - 100]
Clone number	DU 4965
Species	Human
Accession number	P55017.3
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MAELPTTETPGDAT** **LCSGRFTISTLLSSDEPSPPAAYDSSHPSHLTHSSTFCMRTFGYNTIDV** **VPTYEHYANSTQPGEPRKVRPTLADLHSFLKQEGRHL**
Native sequence	Amino acids M1 – L100 (end residue is Q1021) of human NCC. Residue M232 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	*Bam*H1 and *Not*1 sites of pGEX6P-1

Nucleotide Sequence of insert

ggatccATGGCAGAACTGCCCACAACAGAGACGCCTGGGGACGCCACTT
TGTGCAGCGGGCGCTTCACCATCAGCACACTGCTGAGCAGTGATGAGCC
CTCTCCACCAGCTGCCTATGACAGCAGCCACCCCAGCCACCTGACCCAC
AGCAGCACCTTCTGCATGCGCACCTTTGGCTACAACACGATCGATGTGG
TGCCCACATATGAGCACTATGCCAACAGCACCCAGCCTGGTGAGCCCCG
GAAGGTCCGGCCCACACTGGCTGACCTGCACTCCTTCCTCAAGCAGGAA
GGCAGACACCTGtagcggccgc