Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of NKCC1 [1092 – 1195]

Enzyme description:- NKCC1 [1092 - 1195]

Clone number:- DU 30975

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 39, 028.99 daltons
Average Mass 39, 054.16 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.68

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

Clone Data Sheet

NKCC1 [1092 - 1195]

Protein	NKCC1 [1092 - 1195]
Clone number	DU 30975
Species	Human
Accession number	NM_001256461.1
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**IEPYRLHEDDKEQD** **IADKMKEDEPWRITDNELELYKTKTYRQIRLNELLKEHSSTANIIVMSL** **PVARKGAVSSALYMAWLEALSKDLPPILLVRGNHQSVLTFYS**
Native sequence	Amino acids I1092 – S1195 (end) of human NKCC1. Residue I232 of the fusion protein is equivalent to I1092 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

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ggatccATCGAGCCGTACAGACTTCACGAAGATGATAAAGAACAGGACATTGCAGATAAGATGAAA
GAAGATGAGCCTTGGCGAATCACAGATAATGAGCTTGAGCTTTATAAGACTAAGACATATCGACAG
ATCAGGTTAAATGAATTATTAAAGGAGCATTCAAGCACAGCTAACATTATTGTCATGAGTCTTCCA
GTCGCACGGAAAGGCGCTGTGTCCAGTGCTCTGTATATGGCTTGGTTAGAAGCTCTCTCGAAGGAC
CTGCCACCAATCCTCCTGGTTCGTGGGAATCATCAGAGTGTCCTTACCTTCTATTCAtaagcggcc
gc
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