

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of NKCC1 [1 – 288]

Enzyme description:- NKCC1 [1 - 288]

Clone number:- DU 34492

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 55, 753.58 daltons

Average Mass 55, 788.44 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.11

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

NKCC1 [1 - 288]

Protein NKCC1 [1 - 288]

Clone number DU 34492

Species Human

Accession number NM_001256461.1

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERAESMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLVLFQGPLGSMEPRPTAPSSGAPG
LAGVGETPSAAALAAARVELPGTAVSSVPEDAAPASRDGGGVRDEGPAA
AGDGLGRPLGPTPSQSRFQVDLVSENAGRAAAAAAAAAAAAAAAAAAGAGAG
AKQTPADGEASGESEPAKGSEEAKGRFRVNFVDPAASSSAEDSLSDAAG
VGVDGPNVSFQNGGDTVLESGSSLHSGGGGSGHHQHYYYDTHNTYYL
RTFGHNTMDAVPRIDHYRHTAAQLGEKLLRPSLAELHDELEKEPFEDGF
ANGEESTPTRDAVVITYTAESKGVVKGFWI

Native sequence Amino acids M1 – I288 (end) of human NKCC1.
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*I sites of pGEX6P-1

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Nucleotide Sequence of insert

ggatccATGGAGCCGCGGCCACGGCGCCCTCCTCCGGCGCCCCGGGACTGGCCGGGGTTCGGGGAG
ACGCCGTCAGCCGCTGCGCTGGCCGCAGCCAGGGTGGAACTGCCCGGCACGGCTGTGTCCTCGGTG
CCGGAGGATGCTGCGCCCGCGAGCCGGGACGGCGGCGGGGTCCGCGATGAGGGCCCCGCGCGGCC
GGGACGGGCTGGGCAGACCCTTGGGGCCACCCCGAGCCAGAGCCGTTTCCAGGTGGACCTGGTT
TCCGAGAACGCCGGGCGGGCCGCTGCTGCGGCGGCGGCGGCGGCGGCGGCAGCGGCGGCGGCTGGT
GCTGGGGCGGGGGCCAAGCAGACCCCCGCGGACGGGGAAGCCAGCGGCGAGAGCGAGCCGGCTAAA
GGCAGCGAGGAAGCCAAGGGCCGCTTCCGCGTGAAC TTCGTGGACCCAGCTGCCTCCTCGTCGGCT
GAAGACAGCCTGTCAGATGCTGCCGGGGTTCGGAGTCGACGGGCCCCAACGTGAGCTTCCAGAACGGC
GGGACACGGTGCTGAGCGAGGGCAGCAGCCTGCACTCCGGCGGCGGCGGCGGCAGTGGGCACCAC
CAGCACTACTATTATGATACCCACACCAACACCTACTACCTGCGCACCTTCGGCCACAACACCATG
GACGCTGTGCCAGGATCGATCACTACCGGCACACAGCCGCGCAGCTGGGCGAGAAGCTGCTCCGG
CCTAGCCTGGCGGAGCTCCACGACGAGCTGGAAAAGGAACCTTTTGAGGATGGCTTTGCAAATGGG
GAAGAAAGTACTCCAACCAGAGATGCTGTGGTCACGTATACTGCAGAAAGTAAAGGAGTCGTGAAG
TTTGGCTGGATCtaagcggccgc

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