

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of NKCC2 [1 - 174]

Enzyme description:- NKCC2 [1 - 174]

Clone number:- DU 6484

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 45, 985.59 daltons

Average Mass 46, 014.72 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.09

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

NKCC2 [1 - 174]

<u>Protein</u>	NKCC2 [1 - 174]
<u>Clone number</u>	DU 6484
<u>Species</u>	Human
<u>Accession number</u>	Q13621.2
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAESMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSMSLNNSSNVFLDSV PSNTNRFQVSVINENHESSAAADDNTDP PHYEETSFGDEAQRLRISFR PGNQECYDNFLQSGETAKTDASFHAYDSHTNTYYLQTFGHNTMDAVPKI EYYRNTGSISGPKVNRPSLLEIHEQLAKNVAVTPSSADRVANGDGIPGD EQAENKEDDQAGV</p>
<u>Native sequence</u>	Amino acids M1 – V174 (end residue is S1099) of human NKCC2. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX6P-1

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Nucleotide
Sequence of insert

gaattcgATGGATCCATGTCACCTGAACAACCTCTTCCAATGTATTTCTGG
ATTCAGTGCCAGTAATACCAATCGCTTTCAAGTTAGTGTCATAAATGA
GAACCATGAGAGCAGTGCAGCTGCAGATGACAATACTGACCCACCACAT
TATGAAGAAACCTCTTTTGGGGATGAAGCTCAGAAAAGACTCAGAATCA
GCTTTAGGCCTGGGAATCAGGAGTGCTATGACAATTTCTCCAAAGTGG
AGAAACTGCTAAAACAGATGCCAGTTTTTCACGCTTATGATTCTCACACA
AACACATACTATCTACAACTTTTGGCCACAACACCATGGATGCCGTTTC
CCAAGATAGAGTACTATCGTAACACCGGCAGCATCAGTGGGCCCAAGGT
CAACCGACCCAGCCTGCTTGAGATTCACGAGCAACTCGCAAAGAATGTG
GCAGTCACCCCAAGTTCAGCTGACAGAGTTGCTAACGGTGATGGGATAC
CTGGAGATGAACAAGCTGAAAATAAGGAAGATGATCAAGCTGGTGTta
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