

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of KCC1 [872 – 1085]

Enzyme description:- KCC1 [872 - 1085]

Clone number:- DU 30344

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 51966.23 daltons

Average Mass 51, 999.83 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.72

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

KCC1 [872 - 1085]

<u>Protein</u>	KCC1 [872 - 1085]
<u>Clone number</u>	DU 30344
<u>Species</u>	Human
<u>Accession number</u>	NM_005072.4
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAESMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWOATFGGGDHPPKSDLEVLFFQGPLGSRKCRMRIFTVAQMD DNSIQMKKDLAVFLYHLRLEAEVEVEMHNSDISAYTYERTLMMEQRSQ MLRQMRLTKTEREREAQLVKDRHSALRLESLSYDEEDESAVGADKIQMT WTRDKYMTETWDP SHAPDNFRELVHIKPDQSNVRRMHTAVKLNEVIVTR SHDARLVLLNMPGPPRNSEG DENYMEFLEVLTEGLERVLLVRGGGREVI TIYS</p>
<u>Native sequence</u>	<p>Amino acids R872 – S1085 (end) of human KCC1. Residue R232 of the fusion protein is equivalent to R872 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pGEX6P-1

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Nucleotide Sequence of insert

ggatccAGGAAGTGCCGGATGCGCATCTTCACAGTGGCCCAGATGGATGACAACAGCATCCAGATG
AAGAAGGACCTGGCTGTCTTTCTGTACCATCTGCGCCTTGAGGCCGAGGTGGAGGTGGTGGAGATG
CATAACAGTGACATCTCTGCATACACCTACGAGCGGACGCTGATGATGGAGCAGCGGTTCGCAGATG
CTGCGGCAGATGAGACTGACCAAGACTGAGCGGGAGCGAGAAGCCCAGCTGGTCAAGGATCGGCAC
TCGGCCCTGAGGCTGGAGAGCCTGTACTCGGACGAGGAAGATGAGTCTGCAGTGGGGGCTGACAAG
ATCCAGATGACGTGGACCAGGGACAAGTACATGACTGAGACCTGGGACCCAGCCATGCCCCTGAC
AATTTCCGGGAGCTGGTGCACATTAAGCCGGACCAATCCAATGTGCGGCGCATGCACACTGCTGTG
AAGCTCAATGAAGTCATTGTCACGCGCTCCACGACGCCCGCCTGGTTCTCCTAAACATGCCTGGC
CCACCCAGGAACAGTGAGGGCGACGAGAACTACATGGAGTTCCTCGAGGTGCTGACCGAGGGCCTT
GAGCGGGTGCTGTTGGTGCGCGGTGGTGGCCGTGAAGTCATCACCATCTACTCctgaactagtgg
accatcgatgcggccgc

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