

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of KCC2 [1 – 119]

Enzyme description:- KCC2 [1 - 119]

Clone number:- DU 30166

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 39, 804.92 daltons

Average Mass 39, 830.43 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.60

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

KCC2 [1 - 119]

<u>Protein</u>	KCC2 [1 - 119]
<u>Clone number</u>	DU 30166
<u>Species</u>	Human
<u>Accession number</u>	NM_001134771.1
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSMSRRFTVTS LPPAG PARSPDPESRRHSVADPRHLPGEDVKGDGNPKESSPFINSTDTEKGKEY DGKNMALFEEEMDTSPMVSSLLSGLANYTNLPQGSREHEEAENNEGKK KPVQAPR</p>
<u>Native sequence</u>	<p>Amino acids 1 – R119 (end residue S1139) of human KCC2. Residue M232 of the fusion protein is equivalent to M1 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>Bgl</i> 2 and <i>Not</i> 1 sites ligated into <i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX6P-1

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Nucleotide Sequence of insert

ggatctATGAGCCGCAGGTTACAGTCACCTCGCTGCCCCCGCAGGGCCCGCCAGAAGCCCTGAC
CCGGAGTCCCGCCGGCATTTCGGTCGCAGACCCCGCCACCTCCCGGGGAAGACGTCAAAGGTGAT
GGCAACCCCAAGGAAAGCAGTCCCTTCATCAACAGCACCGACACAGAGAAGGAAAGGAGTATGAT
GGCAAGAACATGGCCTTGTTTGAGGAGGAGATGGACACCAGCCCTATGGTGTCTCCTTGCTCAGT
GGCCTGGCCAAC TACACCAACCTGCCCCAGGGAAGTAGGGAGCATGAAGAGGCAGAAAACAATGAG
GGTGGAAAAAAGAAGCCGGTGCAGGCCCCACGctagcggccgc

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