

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of KCC4 [1 – 117]

Enzyme description:- KCC4 [1 - 117]

Clone number:- DU 30637

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 39, 699.62 daltons

Average Mass 39, 725.07 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.87

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

KCC4 [1 - 117]

Protein KCC4 [1 – 117]

Clone number DU 30637

Species Human

Accession number NM_006598.2

Tags N-terminal GST

Bacterially expressed protein MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA
VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSMPTNFTVVPVEAHA
DGGGDETAERTEAPGTPEGPEPERPSPGDGNPRENSPFLNNVEVEQESF
FEGKNMALFEEEMDSNPMVSSLLNKLANYTNLSQGVVEHEEDEESRRRE
AKAPR

Native sequence Amino acids M1 – R117 (end residue S1083) of human KCC4.
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

Nucleotide Sequence of insert

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ggatccATGCCACCAACTTCACCGTGGTGCCCGTGGAGGCTCACGCCGACGGCGGGGACGAG  
ACTGCCGAGCGGACGGAGGCTCCGGGCACCCCGAGGGCCCCGAGCCCGAGCGCCCCAGCCCGGA  
GATGGAAATCCAAGAGAAAACAGCCCATTCCTCAACAATGTCGAGGTGGAACAAGAGAGCTTCTTT  
GAAGGGAAGAACATGGCACTTTTTCGAGGAGGAGATGGACAGTAACCCCATGGTGTCTCGCTGCTC  
AACAAGCTGGCCAACCTACCAACCTGAGCCAGGGCGTGGTGGAGCACGAGGAGGACGAGGAGAGC  
CGGCGGGCGGAGGCCAAGGCTCCGCGCtagcggccgc
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