Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of KCC3 [1 – 175]

Enzyme description:- KCC3 [1 - 175]

Clone number:- DU 30164

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 46, 595.15 daltons
Average Mass 46, 624.83 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.20

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

KCC3 [1 - 175]

Protein	KCC3 [1 – 175]
Clone number	DU 30164
Species	Human
Accession number	NM_00104296.1
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**MASVRFMVTPT** **KIDDIPGLSDTSPDLSSRSSSRVRFSSRESVPETSRSEPMSEMSGATTS** **LATVALDPPSDRTSHPQDVIEDLSQNSITGEHSQLLDDGHKKARNAYLN** **NSNYEEGDEYFDKNLALFEEEMDTRPKVSSLLNRMANYTNLTQGAKEHE** **EAENITEGKKKPTKTPQ**

Native sequence	Amino acids M1 – Q175 (end residue S1141) of human KCC3. Residue M235 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	*Eco*R1 and *Not*1 sites of pGEX6P-1

Nucleotide Sequence of insert

ggatccccggaattcATGGCTTCAGTTCGGTTCATGGTGACACCGACAAAGATCGATGACATTCCA
GGTTTGTCAGACACCAGTCCGGACCTCAGCTCTCGATCTAGTTCCCGAGTAAGATTTAGCTCCCGG
GAAAGCGTGCCTGAAACAAGCCGGAGTGAGCCTATGAGTGAGATGTCTGGGGCCACCACTTCGCTG
GCAACTGTTGCACTGGATCCACCCAGTGACCGGACTTCTCACCCCCAGGATGTCATCGAGGACCTG
AGTCAGAACTCCATCACAGGGGAACACAGCCAACTGTTAGACGACGGACATAAGAAAGCTCGAAAT
GCTTATCTCAATAATTCCAATTATGAAGAAGGAGATGAATATTTTGATAAAAATTTGGCACTCTTT
GAGGAAGAAATGGACACCAGACCGAAGGTGTCTTCCCTCCTCAACCGCATGGCCAATTACACTAAT
CTGACTCAAGGAGCAAAGGAACATGAAGAGGCAGAAAACATCACTGAAGGGAAAAAGAAGCCCACC
AAGACCCCCCAAtagcggccgc