

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of KCC2 [852 – 1116]**

**Enzyme description:-** KCC2 [852 - 1116]

**Clone number:-** DU 30581

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 57, 224.89 daltons

Average Mass 57, 261.51 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 5.69

**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

*Division of Signal Transduction Therapy*

**Clone Data Sheet**

**KCC2 [852 - 1116]**

<b><u>Protein</u></b>	KCC2 [852 - 1116]
<b><u>Clone number</u></b>	DU 30581
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NM_020708.4
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSRKCKMRIFTVAQMD <b>DNSIQMKKDLTTFLYHLRITAEVEVEMHESDISAYTYEKTLMVEQRSQ</b> <b>ILKQMHLLTKNEREREIQSITDESRSIRRKNPANTRLRLNVPEETAGDS</b> <b>EKPEEEEVQLIHDQSAPSCPSSSPSPGEEPEGEGETDPEKVHLTWTKDK</b> <b>SVAEKNKGSPVVSSEGIKDFFSMKPEWENLNQSNVRRMHTAVRLNEVIV</b> <b>KKSRDAKLVLLNMPGPPRRNRNGDENYMEFLEVLTEHLDRVMLVRGGGRE</b> <b>VITIYS</b></p>
<b><u>Native sequence</u></b>	<p>Amino acids R852 – S1116 (end) of human KCC2. Residue R232 of the fusion protein is equivalent to R852 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<b><u>Protease cleavage</u></b>	PreScission ( <u>LEVLFQGP</u> ) residues 221 - 228
<b><u>Cloning sites</u></b>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX6P-1

## *Division of Signal Transduction Therapy*

### Nucleotide Sequence of insert

ggatcccGGAAGTGCAAGATGCGTATCTTCACTGTGGCCAGATGGATGACAATAGCATCCAGATG  
AAGAAGGATCTGACCACATTTCTGTATCATTTACGCATCACTGCGGAGGTCGAGGTGGTGGAGATG  
CATGAGAGCGACATCTCAGCTTACACCTATGAGAAGACGTTGGTGATGGAGCAGCGTCCCAGATC  
CTCAAACAGATGCATTTAACCAAGAATGAGCGGGAGCGGGAGATCCAGAGTATCACAGATGAGTCA  
CGAGGCTCAATCCGGAGAAAGAATCCAGCCAACACGCGGCTCCGCCTGAACGTCCCAGAAGAGACG  
GCTGGTGACAGTGAAGAGAAGCCAGAGGAGGAGGTGCAGCTGATCCACGATCAGAGTGCTCCCAGC  
TGCCCCAGCAGCTCCCCGTCCCCAGGGGAGGAGCCTGAGGGGGAAGGGGAGACAGATCCGGAGAAG  
GTGCATCTCACCTGGACCAAGGACAAGTCGGTGGCAGAGAAGAATAAGGGCCCCAGTCCTGTCTCC  
TCTGAGGGCATCAAGGACTTCTTCAGCATGAAGCCGGAGTGGGAGAACTTGAACCAGTCCAACGTG  
CGGCGCATGCACACGGCCGTGCGGCTGAACGAGGTATCGTGAAGAAATCCCGGGACGCCAAGCTT  
GTTTTGCTCAACATGCCTGGGCCTCCCCGCAACCGCAATGGTGATGAAAACACTACATGGAGTTTCTC  
GAGGTCTCACAGAGCACCTGGACCGGTTGATGCTGGTCCGCGGCGGCGGCCGCGAGGTCATCACC  
ATCTACTCctgagcggccgc

*Division of Signal Transduction Therapy*