University of Dundee

Standard Operating Procedure

Preparation of active SRPK1 [2 – 654]

Enzyme description:- SRPK1 [2 - 654]

Clone number:- DU 967

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 8 mg/L

Calculated molecular mass:- 100, 854 daltons

Purity:- >75 %

Activation protocol:- Constitutively active

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, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- -70 °C

Assay:- Standard filter binding assay

Assay Buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2–mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-
RSRSRSRSRSRSRSR residues 204 – 218 of human ASF-1/SF-2
Final concentration: 300 μM

Specific activity range:- 4000 – 8000 U/mg

University of Dundee

Clone Data Sheet - SRPK1 [2 - 654]

Protein	SRPK1 [2 – 654]
Clone number	DU 967
Species	Human
Accession number	NM_003137
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**ERKVLALQA** **RKKRTKAKKDKAQRKSETQHRGSAPHSESDLPEQEEEILGSDDDEQED** **PNDYCKGGYHLVKIGDLFNGRYHVIRKLGWGHFSTVWLSWDIQGKKFV** **AMKVVKSAEHYTETALDEIRLLKSVRNSDPNDPNREMVVQLLDDFKIS** **GVNGTHICMVFEVLGHHLLKWIIKSNYQGLPLPCVKKIIQQVLQGLDY** **LHTKCRIIHTDIKPENILLSVNEQYIRRLAAEATEWQRSGAPPPSGSA** **VSTAPQPKPADKMSKNKKKKLKKKQKRQAELLEKRMQEIEEMEKESGP** **GQKRPNKQEESESPVERPLKENPPNKMTQEKLEESSTIGQDQTLMERD** **TEGGAAEINCNGVIEVINYTQNSNNETLRHKEDLHNANDCDVQNLNQE** **SSFLSSQNGDSSTSQETDSCTPITSEVSDTMVCQSSSTVGQSFSEQHI** **SQLQESIRAEIPCEDEQEQEHNGPLDNKGKSTAGNFLVNPLEPKNAEK** **LKVKIADLGNACWHKHFTEDIQTRQYRSLEVLIGSGYNTPADIWSTAC** **MAFELATGDYLFEPHSGEEYTRDEDHIALIIELLGKVPRKLIVAGKYS** **KEFFTKKGDLKHITKLKPWGLFEVLVEKYEWSQEEAAGFTDFLLPMLE** **LIPEKRATAAECLRHPWLNS**
Native sequence	Amino acids E2 – S654 (end) of human SRPK1. Residue E2 of the fusion protein is equilivalent to E232 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGPL) at residues 221 – 229
Cloning sites	*Bam*H1 and *Eco*R1 site of pGEX6P-1