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Purification of SRC kinase

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Elias Adriaenssens[1]

[1]Sascha Martens lab, University of Vienna, Max Perutz Labs - Vienna

Elias Adriaenssens
Sascha Martens lab, University of Vienna, Max Perutz Labs - ...

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We use this protocol and it's working

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Abstract

This protocol details the purification of SRC kinase.

Materials

Wash buffer:

	A	B
	Tris-HCl, pH 7.4	50 mM
	NaCl	300 mM
	Glycerol	5%
	DTT	1 mM

Salt wash buffer:

	A	B
	Tris-HCl, pH 7.4	50 mM
	NaCl	700 mM
	Glycerol	5%
	DTT	1 mM

SEC buffer:

	A	B
	Tris-HCl, pH 7.4	25 mM
	NaCl	300 mM
	DTT	1 mM

25ml Lysis buffer:

	A	B
	Tris- HCl, pH 7.4	50 mM
	NaCl	300 mM
	DTT	1 mM
	MgCl2	2 mM
	β-mercaptoethanol	2 mM
	Glycerol	5%
	Triton X- 100	1%
	Benzonase	1µl

Purification

10h 45m

1 To purify SRC (WT or Y530F), we purchased gene-synthesized codon-optimized Src-TEV-GST in a pFastBac-Dual vector from Genscript (available from Addgene) for expression in insect cells. Use the constructs to generate bacmid DNA, using the Bac-to-Bac system, by amplification in DH10BacY cells 25.

2 After the bacmid DNA was verified by PCR for insertion of the transgene, purify bacmid DNA for transfection into Sf9 insect cells (12659017, Thermo Fisher, RRID:CVCL_0549).

3 To this end, mix 2500 ng of plasmid DNA with FuGene transfection reagent (Promega) and transfect and seed 1 million Sf9 cells in a 6 well plate.

4 About 7 days after transfection, harvest the V0 virus and use to infect 40 mL of 1 million cells per ml of Sf9 cells.

5 Closely monitor the viability of the cultures and upon the decrease in viability and confirmation of yellow fluorescence, collect the supernatant after centrifugation and store this as V1 virus. For expressions, infect 1 L of Sf9 cells, at 1 million cells per ml, with 1 mL of V1 virus.

6 When the viability of the cells decreased to 90-95%, collect the cells by centrifugation.

7 Wash cell pellets with 1x PBS and flash-frozen in liquid nitrogen.

8 Store the pellets at -80 °C .

9 For purification of SRC(Y530F), resuspend pellets in 25 ml lysis buffer, 1 µl benzonase (Sigma), complete EDTA-free protease inhibitors (Roche), CIP protease inhibitor (Sigma)).

25ml Lysis buffer:

	A	B
	Tris- HCl, pH 7.4	50 mM
	NaCl	300 mM

	A	B
	DTT	1 mM
	MgCl2	2 mM
	β-mercaptoethanol	2 mM
	Glycerol	5%
	Triton X- 100	1%
	Benzonase	1µl

10 Homogenize and clear cells with a douncer and lysates by centrifugation at 18000 rpm, 4°C, 00:45:00 in a SORVAL RC6+ centrifuge with an F21S-8x50Y rotor (Thermo Scientific). 45m

11 Collect and incubate the supernatant with pre-equilibrated Glutathione Sepharose 4B beads (GE Healthcare) for 02:00:00 at 4 °C with gentle shaking to bind SRC(Y530F)-TEV-GST. 2h

12 Centrifuge the samples to pellet the beads and remove the unbound lysate.

13 Wash the beads twice with wash buffer, once with high salt wash buffer, and two more times with wash buffer.

Wash buffer:

	A	B
	Tris-HCl, pH 7.4	50 mM
	NaCl	300 mM
	Glycerol	5%
	DTT	1 mM

Salt wash buffer:

	A	B
	Tris-HCl, pH 7.4	50 mM
	NaCl	700 mM
	Glycerol	5%
	DTT	1 mM

14 Incubate beads Overnight with TEV protease in wash buffer at 4 °C . 8h

Wash buffer:

	A	B
	Tris-HCl, pH 7.4	50 mM
	NaCl	300 mM
	Glycerol	5%
	DTT	1 mM

15 After release the proteins from the beads by the TEV protease, collect the supernatant after centrifugation of the beads.

16 Wash the beads twice with 4 mL of wash buffer, and collect the supernatant.

17 Filter and pool the supernatant fractions through a 0.45 µm syringe filter, and concentrate with a 30 kDa cut-off Amicon filter (Merck Millipore).

18 Load the proteins onto a pre-equilibrated Superdex 200 Increase 10/300 GL column (Cytiva).

19 Elute the proteins with SEC buffer.

SEC buffer:

	A	B
	Tris-HCl, pH 7.4	25 mM
	NaCl	300 mM
	DTT	1 mM

20 Analyse fractions by SDS-PAGE and Coomassie staining.

21 Pool the fractions containing purified SRC(Y530F).

22 After concentrating the purified protein, aliquot the protein and snap-frozen in liquid nitrogen.

23 Store proteins at -80 °C.