Aug 29, 2024

Purification of CK2 kinase complex

DOI
dx.doi.org/10.17504/protocols.io.eq2lyww1evx9/v1

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 - ...

DOI: dx.doi.org/10.17504/protocols.io.eq2lyww1evx9/v1

Protocol Citation: Elias Adriaenssens 2024. Purification of CK2 kinase complex. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.eq2lyww1evx9/v1**

Protocol status: Working
We use this protocol and it's working

Created: May 23, 2024

Last Modified: August 29, 2024

Protocol Integer ID: 101123

Keywords: ASAPCRN

Funders Acknowledgement:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-000350
Marie Skłodowska-Curie MSCA Postdoctoral fellowship
Grant ID: 101062916

Abstract

This protocol details the purification of CK2 kinase complex.

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 procedure

10h 45m

1 To purify the CK2 kinase complex, subclone GST-TEV-CK2α together with CK2β in a pFastBac-Dual vector (available from Addgene) and GST-TEV-CK2α' together with CK2β in a pFastBac-Dual vector (available from Addgene) for co-expression in insect cells.

2 Use the constructs to generate bacmid DNA, using the Bac-to-Bac system, by amplification in DH10BacY cells [25].

3 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).

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

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

6 Closely monitor the viability of the cultures and upon the decrease in viability and confirmation of yellow fluorescence, collect the supernatant after centrifugation and stored this as V1 virus.

7 For expressions, infect 1 L of Sf9 cells (12659017, Thermo Fisher, RRID:CVCL_0549), at 1 million cells per ml, with 1 mL of V1 virus for GST-TEV-CK2α/CK2β and 1 mL of V1 virus for GST-TEV-CK2α'/CK2β.

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

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

10 Store pellets at -80 °C .

11 For purification of the CK2 kinase complex, resuspend pellet 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
	DTT	1 mM
	MgCl2	2 mM
	β-mercaptoethanol	2 mM
	Glycerol	5%
	Triton X- 100	1%
	Benzonase	1µl

12 Homogenize and clear the 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

13 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 the CK2 complex. 2h

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

15 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

	A	B
	NaCl	700 mM
	Glycerol	5%
	DTT	1 mM

16 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

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

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

19 Pool and filter the supernatant fractions through a 0.45 µm syringe filter, and concentrated with a 10 kDa cut-off Amicon filter (Merck Millipore).

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

21 Elute the proteins with SEC buffer.

SEC buffer:

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

22 Analyze fractions by SDS-PAGE and Coomassie staining.

23 Pool the fractions containing purified CK2α/CK2α'/CK2β.

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