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Purification of CK2 kinase complex

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We use this protocol and it's working

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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
	MgCl ₂	2 mM
	β-mercaptoethanol	2 mM



	A	B
	Glycerol	5%
	Triton X- 100	1%
	Benzonase	1μl



Purification procedure

10h 45m

- 1 To purify the CK2 kinase complex, subclone GST-TEV-CK2 α together with CK2 β in a pFastBac-Dual vector (available from Addgene) and GST-TEV-CK2 α' together with CK2 β 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-CK2 α /CK2 β and  1 mL of V1 virus for GST-TEV-CK2 α' /CK2 β .
- 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 μ l 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
MgCl ₂	2 mM
β -mercaptoethanol	2 mM
Glycerol	5%
Triton X- 100	1%
Benzonase	1 μ l

- 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-8 $\times$ 50Y 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



	A	B
	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

- 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 CK2 α /CK2 α' /CK2 β .

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