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ATP synthase activity assay (radioactive)

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

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Abstract

This protocol describes how to detect synthesis of [α^{32}P]ATP from [α^{32}P]ATP by recombinant KaiC proteins. Radioactive nucleotides are separated via thin layer chromatography using TLC PEI Cellulose F plates as stationary phase and LiCl as soluble phase. The principle of this method is based on Egli *et al.* (Egli M, Mori T, Pattanayek R, Xu Y, Qin X, Johnson CH.2012. Dephosphorylation of the core clock protein KaiC in the cyanobacterial KaiABC circadian oscillator proceeds via an ATP synthase mechanism. Biochemistry 51:1547-58.)

Materials

MATERIALS

- Magnesium chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich)**
- [y-32P]ATP (3000 Ci/mmol) **Hartmann Analytic GmbH Catalog #**SRP-301
- Tris(hydroxymethyl)aminomethane **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**252859-500G
- NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**53014
- EDTA
- Lithium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**793620
- Adenosin-5-triphosphate disodium salt (ATP) **Carl Roth Catalog #**HN35.1
- TLC PEI Cellulose F plates **Merck Millipore (EMD Millipore) Catalog #**1055790001
- Adenosine 5′-diphosphate sodium salt (ADP) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A2754-100MG
- [alpha-P32]ADP 6000 Ci/mmol 10 mCi/ml **Hartmann Analytic GmbH Catalog #**SRP-227

preparation

1
- express and purify KaiC
- optional: phosphorylate or dephosphorylate KaiC prior to analysis

incubation

2
- prepare a 25 µl mastermix with 3 µM KaiC in ATP synthesis buffer (20 mM Tris/HCl (pH8), 150 mM NaCl, 0.5 mM EDTA, 5 mM MgCl2, 0.5 mM ATP)
- add 2 ul [α-32P]ADP (= 20 µCi)
- freeze one 10 µl aliquot at -20 °C (0h, -20 °C)
- incubate one 10 µl aliquot for 2 hours at 30 °C
- as a control, perform the same reaction but in the presence of 0.5 mM (non-radioactive) ADP
- in total you will have 4 samples for every KaiC protein of interest:

	sample	0.5 mM (non-radioactive) ADP	incubation for 2h at
	control 0h	+	-20 °C
	control 2h	+	30 °C
	sample 0h	-	-20 °C
	sample 2h	-	30 °C

thin layer chromatography

3
- spin down briefly and dilute samples 1:20 in H2O
- spot 0.5 µl of the diluted reaction mixtures and [α-32P]ADP and [γ-32P]ATP (as size controls) onto a TLC PEI Cellulose F plates (Merck Millipore) - place the spots approx 1-2 cm from the bottom of the plate, mark the level with a pencil
- wait until spots are dried
- pour a small amount of 1 M LiCl as mobile phase in a thin layer chromatography chamber (the solvent level must be lower than the distance of your spots from the bottom of the TLC plate)
- close the lid and wait a few minutes

- place the TLC plate in the chamber, close the lid and allow the mobile phase to be drawn up the plate (without reaching the end of the TLC plate)
- remove and dry the TLC plate

detection and analysis

4

- expose dried plates to an autoradiography screen and detect signals using for example a Personal Molecular Imager FX system (Bio-Rad)
- analyze the samples (for example with ImageLab software (Bio-Rad))
- for each lane determine the signal intensity of all detectable spots
- calculate the relative intensity of [α-32P]ATP as percentage of all signals in the corresponding lane
- for normalization substract the relative [α-32P]ATP intensity of the -20 °C sample containing 0.5 mM ADP from the relative [α-32P]ATP intensity in the other samples