ATP synthase activity assay (radioactive)

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1 Works for me

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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

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

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