JUL 11, 2019

OPEN ACCESS

DOI:
[dx.doi.org/10.17504/protocols.io.48qgzvw](https://doi.org/10.17504/protocols.io.48qgzvw)

External link:
<https://doi.org/10.1128/JB.00478-19>

Protocol Citation: Anika Wiegard, Christin Köbler, Katsuaki Oyama, Anja K. Dörrich, Chihiro Azai, Kazuki Terauchi, Annegret Wilde, Ilka Maria IM Axmann 2019. ATP synthase activity assay (radioactive). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.48qgzvw>

MANUSCRIPT CITATION: Wiegard A, Köbler C, Oyama K, Dörrich AK, Azai C, Terauchi K, Wilde A, Axmann IM, Array. Journal of Bacteriology 202(4). doi: [10.1128/JB.00478-19](https://doi.org/10.1128/JB.00478-19)

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

ATP synthase activity assay (radioactive)

Journal of Bacteriology

Anika Wiegard¹, Christin Köbler², Katsuaki Oyama³, Anja K. Dörrich⁴, Chihiro Azai^{5,3}, Kazuki Terauchi^{5,3}, Annegret Wilde², Ilka Maria IM Axmann⁶

¹Heinrich-Heine Universität Düsseldorf;

²Institute of Biology III, Faculty of Biology, University of Freiburg, 79104 Freiburg, Germany;

³Graduate School of Life Sciences, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan;

⁴Institute for Microbiology and Molecular Biology, Justus-Liebig University, 35392 Giessen, Germany;

⁵College of Life Sciences, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan;

⁶Institute for Synthetic Microbiology, Cluster of Excellence on Plant Sciences (CEPLAS), Heinrich Heine University Duesseldorf, 40225 Duesseldorf, Germany

Axmann Lab

CyanoWorld

Anika Wiegard

Heinrich-Heine Universität Düsseldorf

ABSTRACT

This protocol describes how to detect synthesis of [$\alpha^{32}\text{P}$]ATP from [$\alpha^{32}\text{P}$]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.)

Created: Jul 06, 2019

MATERIALS

Last Modified: Jul 11, 2019

PROTOCOL integer ID:
25584

Keywords: ATP synthesis,
thin layer chromatography,
TLC, KaiC

MATERIALS

⊗ Magnesium chloride hexahydrate **Sigma Aldrich**

⊗ [γ - 32 P]ATP (3000 Ci/mmol) **Hartmann Analytic GmbH Catalog #SRP-301**

⊗ Tris(hydroxymethyl)aminomethane **Sigma Aldrich Catalog #252859-500G**

⊗ NaCl **Sigma Aldrich Catalog #53014**

⊗ EDTA **Contributed by users**

⊗ Lithium chloride **Sigma Aldrich Catalog #793620**

⊗ Adenosin-5-triphosphate disodium salt (ATP) **Carl Roth Catalog #HN35.1**

⊗ TLC PEI Cellulose F plates **Merck Millipore Catalog #1055790001**

⊗ Adenosine 5'-diphosphate sodium salt (ADP) **Sigma Aldrich Catalog #A2754-100MG**

⊗ [α - 32 P]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 μ l mastermix with 3 μ M 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 μ l [α - 32 P]ADP (= 20 μ Ci)
 - freeze one 10 μ l aliquot at -20 °C (0h, -20 °C)
 - incubate one 10 μ l 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 subtract 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