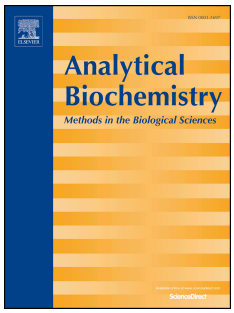
Accepted Manuscript



A rapid fluorescent method for the real-time measurement of poly(ADP-ribose) polymerase 1 activity

T.A. Kurgina, R.O. Anarbaev, M.V. Sukhanova, O.I. Lavrik

PII: S0003-2697(17)30548-1

DOI: 10.1016/j.ab.2017.12.033

Reference: YABIO 12891

To appear in: *Analytical Biochemistry*

Received Date: 7 August 2017

Revised Date: 27 December 2017

Accepted Date: 30 December 2017

Please cite this article as: T.A. Kurgina, R.O. Anarbaev, M.V. Sukhanova, O.I. Lavrik, A rapid fluorescent method for the real-time measurement of poly(ADP-ribose) polymerase 1 activity, *Analytical Biochemistry* (2018), doi: 10.1016/j.ab.2017.12.033.

A rapid fluorescent method for the real-time measurement of poly(ADP-ribose)polymerase 1 activity

Kurgina[1,2‡] T.A., Anarbaev[1,2‡] R.O., Sukhanova[1] M.V., Lavrik[1,2,3*] O.I.

[1]*Institute of Chemical Biology and Fundamental Medicine, Lavrentiev av. 8, 630090 Novosibirsk, Russia; Fax*: +73833333677; Email: lavrik@niboch.nsc.ru

[2]*Department of Natural Sciences, Novosibirsk State University, 2 Pirogov Street, 630090 Novosibirsk, 630090, Russia*

[3]*Depatment of Physico-Chemical Biology and Biotechnology, Altai State University, 656049 Barnaul, Russia*

* To whom correspondence should be addressed. Tel: +7 383 363 5196; Fax: +7 383 363 5153; E-mail: lavrik@niboch.nsc.ru

‡ These authors contributed equally to this work.

Abstract

Poly(ADP-ribose) polymerase 1 (PARP1) is a key enzyme that regulates important cellular processes, including DNA repair. PARP1 binds to a DNA damage site and synthesizes poly(ADP-ribose) chains (PARs), which serve as a signal of DNA damage for other DNA repair enzymes. PARP1 is a recognized target for the development of anti-cancer drugs. In this work, a method is developed that makes it possible to investigate the complex formation of PARP1 with DNA as well as its dissociation by detecting the fluorescence anisotropy of this complex during the poly(ADP-ribose) synthesis. The method allows investigation of the inhibition of PARP1 activity in the presence of its inhibitors. In this work, we demonstrated that PARP1is activated by DNA duplexes containing a damage and a fluorophore at the 3'-end of one of the DNA duplex chains. The effects of the clinical inhibitor olaparib on the activity of PARP1 was studied. It was shown that olaparib has no influence on the binding of PARP1 to the model DNA structures used, but it significantly inhibits the poly(ADP-ribosyl)ation of PARP1. The proposed convenient method for the real-time determination of the PARP1 activity can be used to screen PARP1 inhibitors with the calculation of quantitative inhibition parameters.

***Key Words*:** Poly(ADP-ribose) polymerase 1; Enzyme activity; Assay; Fluorescence anisotropy; Real-time detection.