SZABO SCANDIC

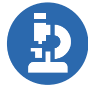
Part of Europa Biosite

Produktinformation

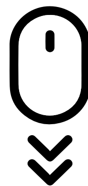
Forschungsprodukte & Biochemikalien

Zellkultur & Verbrauchsmaterial

Diagnostik & molekulare Diagnostik

 Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!



Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The PARG Fluorogenic Assay Kit is a high-throughput, homogeneous 96-well assay designed to measure the hydrolase activity of Poly (ADP-ribose) glycohydrolase (PARG) for screening and profiling applications, using a simple and straightforward fluorogenic assay. The PARG Fluorogenic Assay Kit contains enough purified recombinant PARG enzyme, substrate, and assay buffer for 100 enzyme reactions.

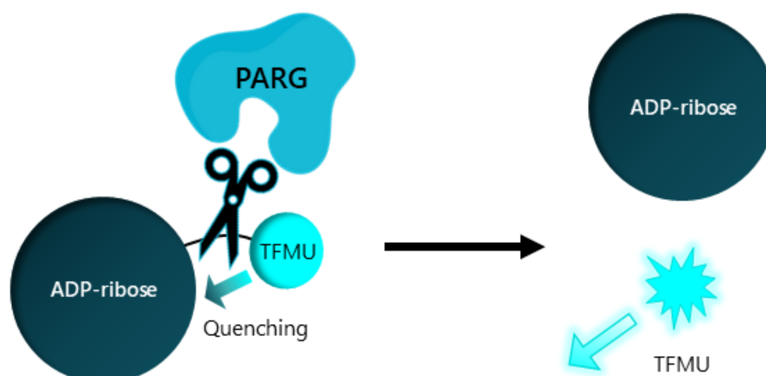

Figure 1: Illustration of the assay principle.

PARG is incubated with a fluorogenic ADP-ribose substrate in which the fluorophore is quenched by the presence of the ribose. PARG-mediated hydrolysis of the substrate between the ribose and the fluorochrome releases fluorescence that can be detected at λ=502 nm (excitation at λ=385 nm). Fluorescence intensity is directly proportional to PARG hydrolase activity.

Background

Poly (ADP-ribose) glycohydrolase (PARG) is a catabolic enzyme involved in the degradation of PARylated chains, releasing ADP-ribose and oligo(ADP-ribose) chains. PAR homeostasis is regulated by the family of PAR polymerases (PARPs) and PARG in response to cellular stress conditions such as DNA damage response (DDR). PARG activity is linked to cellular responses in inflammation, ischemia, stroke, and cancer. PARG is overexpressed in breast cancer and associated with tumor growth and survival. Decrease in PARG activity can potentiate the effect of current cancer therapies, such as chemotherapy and radiation, making PARG inhibition with selective inhibitors a promising approach in cancer and immunotherapy.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

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Supplied Materials

Catalog #	Name	Amount	Storage
101726	PARG, Full Length, His-Tag*	0.5 µg	-80°C
	Fluorogenic PARG Substrate (1 mM)	25 µl	-20°C
	Stock PARG Hydrolase Buffer	1.2 ml	-20°C
	0.5 M DTT	200 µl	-20°C
	PDD00017273 (10 mM)**	20 µl	-20°C
79685	96-well microplate, black	1 plate	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube.*
***The kit includes the PARG inhibitor PDD00017273 as a PARG activity control.*

Materials Required but Not Supplied

- Fluorescence plate reader capable of excitation at 385 nm and detection at 502 nm
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed. ***Avoid multiple freeze/ thaw cycles!***

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

- The final concentration of DMSO in the assay should not exceed 1%.
- Compounds that emit fluorescence near λ=502 nm when excited at λ=385 nm can interfere with results.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include a “Blank”, “Positive Control” and “Inhibitor Control”.
- If the assay plate is going to be used more than once, prepare enough reagents for this portion of the assay and aliquot the remaining undiluted reagents into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C or at -20°C as appropriate.

1) Thaw **Stock PARG Hydrolase Buffer** at Room Temperature (RT).

2) Prepare **Diluted PARG Hydrolase Buffer** by diluting **Stock PARG Hydrolase Buffer** 5-fold with distilled water.

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3) Dilute **0.5 M DTT** 500-fold in Diluted PARG Hydrolase Buffer to make a 1 mM concentration. This is now the **Assay Buffer**.

4) Thaw **PARG enzyme** on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube.

5) Calculate the amount of PARG required for the assay (20 µl/well) and dilute enzyme to **0.25 ng/µl** with **Assay Buffer**. Store remaining undiluted PARG enzyme at -80°C in single-use aliquots.

   Note: PARG enzyme is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. ***Do not re-use the diluted enzyme.***

6) Add 20 µl of diluted PARG to the "Positive Control" and "Test Inhibitor" wells.

7) Add 20 µl of Assay Buffer to the "Blank" wells.

8) Prepare the Test Inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   8.1 If the Test Inhibitor is water-soluble, prepare serial dilutions 10-fold more concentrated than the desired final concentrations using the Assay Buffer. Assay Buffer is the Diluent Solution.

   OR

   8.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO, at a concentration 100-fold higher than the highest desired final concentration, then dilute the inhibitor 10-fold in Assay Buffer to prepare the highest concentration of the 10-fold intermediate serial dilutions. The concentration of DMSO is now 10%.

   Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in Assay Buffer to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   The final concentration of DMSO should not exceed 1%.

9) Add 5 µl of the Test Inhibitor solution to each well designated as "Test Inhibitor".

10) Add 5 µl of Diluent Solution to the "Positive Control" and "Blank" wells.

11) Prepare the Inhibitor Control by diluting PDD00017273 (10 mM) 100-fold in 100% DMSO to make a 100 µM solution. Dilute 10-fold in Assay Buffer to make a 10 µM solution (the DMSO amount is now 10%).

12) Add 5 µl of 10 µM PDD00017273 to the "Inhibitor Control" wells.

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13) Incubate at Room Temperature for 15 minutes.

    Note: We strongly recommend pre-incubation of the enzyme with the inhibitor before adding the substrate.

14) Thaw **Fluorogenic PARG Substrate (1 mM)** on ice.

15) Prepare **Substrate Solution** (10 µM) by diluting **Fluorogenic PARG Substrate (1 mM)** 100-fold in Assay Buffer. You will need 25 µl/well. Dilute only enough substrate required for the assay. Store remaining Fluorogenic PARG Substrate (1 mM) at -20°C in single-use aliquots.

16) Initiate the reaction by adding 25 µl of Substrate Solution to all wells.

17) Incubate at Room Temperature for 1 hour protected from light.

	Blank	Positive Control	Inhibitor Control	Test Inhibitor
Diluted PARG enzyme (0.25 ng/µl)	-	20 µl	20 µl	20 µl
Test Inhibitor	-	-	-	5 µl
Diluted PDD00017273	-	-	5 µl	-
Diluent Solution	5 µl	5 µl	-	-
Assay Buffer	20 µl	-	-	-
Incubate 15 minutes at Room Temperature				
Substrate Solution (10 µM)	25 µl	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

18) Measure fluorescence in a plate reader capable of excitation at 385 nm and emission at 502 nm.

19) “Blank” value should be subtracted from all other values.

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Example Results

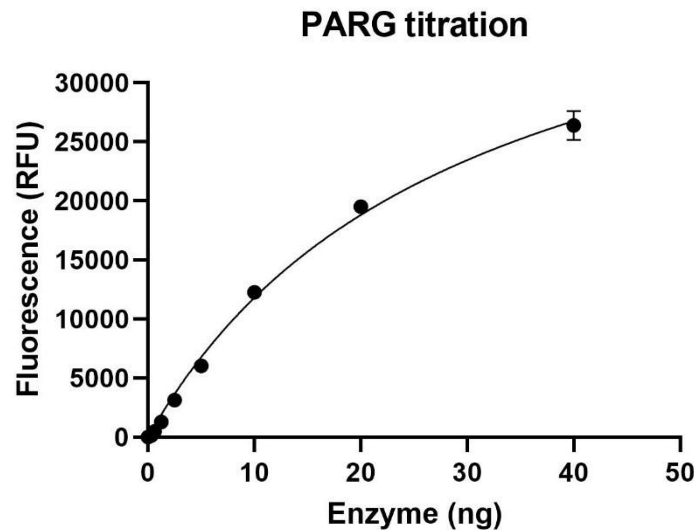

Figure 2: Titration of PARG.
Increasing amounts of enzyme were incubated with 5 µM Fluorogenic PARG substrate. Fluorescence was measured using a Bio-Tek microplate reader.

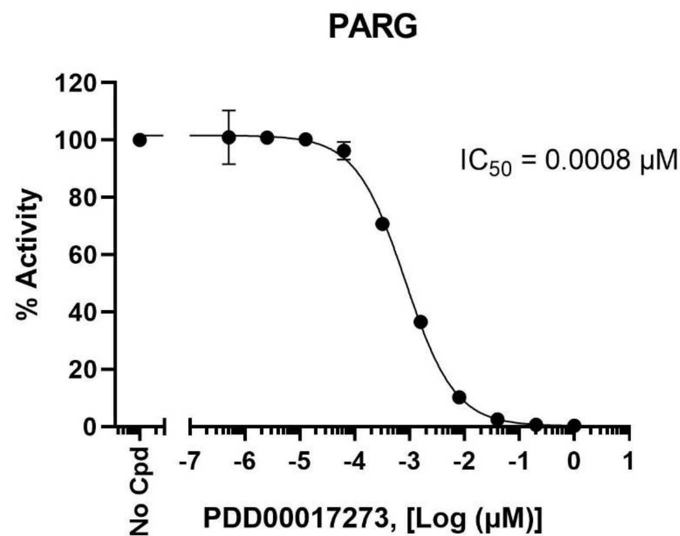

Figure 3: PARG inhibition by PDD00017273.
PARG activity was measured in the presence of increasing concentrations of PDD00017273. Fluorescence was measured using a Bio-Tek microplate reader.

For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For further questions, please email support@bpsbioscience.com

References

1. Marques, M. *et al*. 2019 *Oncogene* 38 (12): 2177-2191.
2. James, D. I. *et al*., 2016 *ACS Chem Biol* 11 (11): 3179-3190.
3. Drown, B. S. *et al*., 2018 *Cell Chem Bio* 25 (12): 1562-1570.

Related Products

Products	*Catalog #*	*Size*
PARG Fluorogenic Assay Kit	78858	384 reactions
PARP1 Colorimetric Assay Kit	80580	96 reactions
PARP1 Homogeneous Assay Kit	78438	384 reactions
PARP1, FLAG-Avi-Tag Recombinant	80521	20 µg
PARP1, GST-Tag Recombinant	80501	20 µg

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