Description

The ARH3 Fluorogenic Assay Kit is a high-throughput, homogeneous 96-well assay designed to measure the hydrolase activity of ARH3 (ADP-ribosylhydrolase 3) for screening and profiling applications, using a simple and straightforward fluorogenic assay. The ARH3 Fluorogenic Assay Kit contains enough purified recombinant ARH3 enzyme, substrate, and assay buffer for 100 enzyme reactions. The kit includes the ARH3 inhibitor ADP-HPD as an ARH3 activity control.

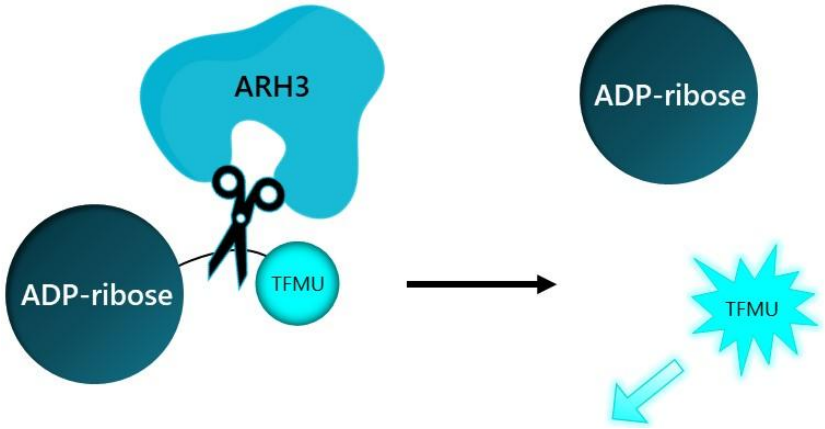

Figure 1: Illustration of the assay principle.

ARH3 is incubated with a fluorogenic ADP-ribose substrate in which the fluorophore is quenched by the presence of the ribose. ARH3-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 ARH3 hydrolase activity.

Background

ARH3, also known as ADP-ribosyl-acceptor hydrolase 3 or ADPRS, is part of the DNA damage response (DDR) machinery. It removes ADP-ribose from serine residues in a Mg^{2+}- dependent manner. It acts sequentially to PARG (poly(ADP-ribose) glycohydrolase) and it has a protective role by decreasing the levels of ADP-ribosylation in the cell, which stops mitochondria from releasing PAR-driven AIF (apoptosis inducing factor). Mutations that result in loss of function of this protein lead to CONDSIAS (stress-induced childhood-onset neurodegeneration with variable ataxia and seizures), a disease with multiple clinical expressions. Patients can accumulate mono(ADP-ribose) on core histones (scars) and have reduced levels of H3K9 acetylation. *In vitro* these scars were shown to decrease if PARP was inhibited. The development and use of ARH3 inhibitors will provide a better understanding of the DNA damage response pathway and opens new therapeutic avenues.

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
101852-KC1	ARH3, His-Tag*	≥ 1 µg	-80°C
84477-KC25	DP-01 Substrate (1 mM)	25 µl	-20°C
82522-KC1.2	DP-01 Buffer	1.2 ml	-20°C
82735-KC200	0.5 M DTT	200 µl	-20°C
84479-KC10	ADP-HPD (1 mM)	10 µl	-20°C
79685	96-well microplate, black	1 plate	Room Temp

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- Fluorescence plate reader capable of excitation at λ=385 nm and detection at λ=502 nm
- Adjustable micropipettor and sterile tips
- Orbital shaker

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

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 are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound on the assay results.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include a “Blank”, “Positive Control” and “Inhibitor Control”.
- We recommend using ADP-HPD (#84479-KC10) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1x, 1x and 10x the IC_{50} value shown in the validation data below.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

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1. Thaw **DP-01 Buffer** at Room Temperature (RT).

2. Prepare **Diluted DP-01 Buffer** by diluting **DP-01 Buffer** 5-fold with distilled water.

3. Dilute **0.5 M DTT** 500-fold (to final 1 mM) with Diluted DP-01 Buffer to prepare the ready to use **Assay Buffer**.

4. Thaw **ARH3 enzyme** on ice. Briefly spin the tube containing the enzyme to recover its full content.

5. Dilute the enzyme to **0.25 ng/µl** with **Assay Buffer** (20 µl/well).

6. Add 20 µl of **diluted ARH3** to the "Positive Control", "Inhibitor 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 Assay Buffer.

   For positive and negative controls use Assay Buffer (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).

   Note: 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 following the guidelines provided for the Test inhibitor (see above) (5 µl/well).

12. Add 5 µl of diluted ADP-HPD to the "Inhibitor Control" wells.

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13. Incubate at RT for 15 minutes.

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

14. Thaw **DP-01 Substrate (1 mM)** on ice.

15. Prepare a **Substrate Solution (10 µM)** by diluting **DP-01 Substrate (1 mM)** 100-fold with Assay Buffer. You will need 25 µl/well.

    Note: Dilute only enough substrate required for the assay. Store remaining DP-01 Substrate (1 mM) at -20°C in single-use aliquots (minimum volume of 5 µl/ aliquot).

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

17. Incubate at RT for 1 hour protected from light.

	Blank	**Positive Control**	**Inhibitor Control**	**Test Inhibitor**
Diluted ARH3 enzyme (0.25 ng/µl)	-	20 µl	20 µl	20 µl
Assay Buffer	20 µl	-	-	-
Test Inhibitor	-	-	-	5 µl
Diluted ADP-HPD	-	-	5 µl	-
Diluent Solution	5 µl	5 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 λ=385 nm and emission at λ=502 nm.

19. "Blank" value should be subtracted from all other values.

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Example Results

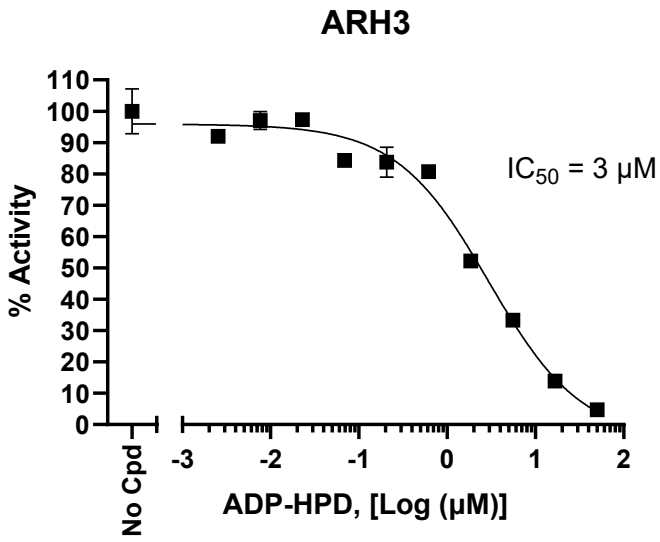

Figure 2: ARH3 inhibition by ADP-HPD.
ARH3 activity was measured in the presence of increasing concentrations of ADP-HPD. Fluorescence was measured using a Bio-Tek microplate reader. Results are expressed as percentage of activity (in which activity in absence of inhibitor was set at 100%).

Data shown is representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References
Rack J.G.M., *et al.*, 2018 *Cell Chem Biol* 25 (12): 1533-1546.
Drown B. S., *et al.*, 2018 *Cell Chem Biol* 25 (12): 1562-1570.
Liu X., *et al.*, 2020 *J Biol Chem* 295 (40): 13838-13849.
Hanzlikova H., *et al.*, 2020 *Nat Commun*. 11(1):3391.

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