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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The PADI4 Fluorogenic Assay Kit is a fluorogenic assay designed to measure the enzymatic activity of PADI4 (protein-arginine deiminase type-4) for screening and profiling applications. This assay uses o-phthalaldehyde and DTT (dithiothreitol) as detection reagents. The assay kit comes in a convenient 96-well format, with enough purified recombinant PADI4, BAEE (Nα-Benzoyl-L-arginine ethyl ester hydrochloride), EDTA (Ethylenediaminetetraacetic acid), o-phthalaldehyde, and assay buffer for 100 reactions.

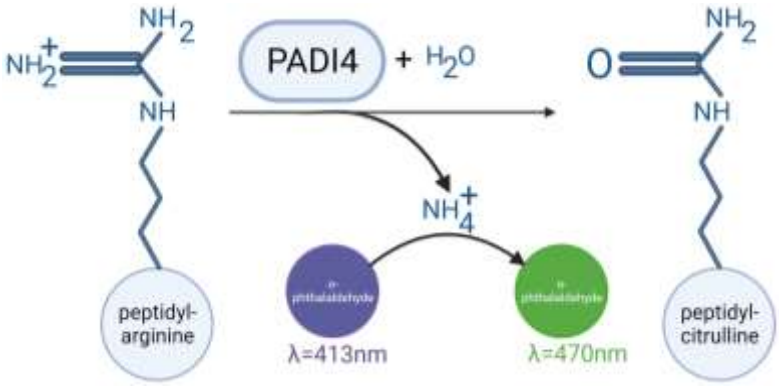

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Figure 1: Assay Principle. Peptidyl-arginine is converted into peptidyl-citrulline by PADI4, releasing NH_4^+. NH_4^+ reacts with DTT-reduced o-phthalaldehyde. O-phthalaldehyde can then fluoresce at 470 nm, and the signal is proportional to the activity of PADI4.

Background

PADI4, also known as peptidylarginine deiminase 4, is a hydrolase that catalyzes the conversion of peptidylarginine to citrulline, a process known as citrullination. PADI4 plays a role in several cellular functions, including gene expression, cell differentiation, and apoptosis. It is involved in immune responses and inflammation through granulocyte and macrophage development and thus contributes to diseases such as RA (rheumatoid arthritis). Dysfunction of PADI4 has also been linked to multiple sclerosis (MS), Alzheimer's disease (AD) and cancer. It contributes to cancer by several possible mechanisms such as epithelial-to-mesenchymal transition (EMT), apoptosis, and formation of neutrophil extracellular traps (NETs). A better understanding of the contribution of PADI4 to cancer development and inflammation, combined with development of inhibitors, will result in new therapeutic avenues.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
50154	PADI4, FLAG-Tag*	40 µg	-80°C
	PADI Assay Buffer	10 ml	-20°C
	0.5 M EDTA	1.5 ml	-20°C
	100 mM BAEE	500 µl	-80°C
	100 mM O-phthalaldehyde (OPT)	1.5 ml	+4 °C
	0.5 M DTT	200 µl	-80°C
	100 mM Phosphate Buffer, pH 7.8	20 ml	Room Temperature
79685	Black 96-well plate	2	Room Temperature

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

Materials Required but Not Supplied

- Ethanol
- Fluorescence plate reader capable of measurement at λex413/λem470 nm
- Adjustable micropipettor and sterile tips
- 37°C incubator

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%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include “Blank”, “Positive Control” and “Test Inhibitor” conditions.

1. Thaw **PADI Assay Buffer** and **100 mM BAEE.**

2. Prepare 1x Assay Buffer by diluting **0.5 M DTT** 200-fold with PADI Assay Buffer.

3. Prepare a Master Mix (25 µl/well): N wells x (20 µl of 1x Assay Buffer + 5 µl of 100 mM BAEE).

4. Add 25 µl to every well.

5. 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.

   5.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in 1x Assay Buffer 10-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use 1x Assay Buffer (Diluent Solution).

   OR

   5.2. If the Test inhibitor is soluble in DMSO: Prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x Assay Buffer to prepare the highest concentration of the 10-fold intermediate 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 1x Assay Buffer to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in 1x 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%.

6. Add 5 µl of Test Inhibitor to each well labeled “Test Inhibitor”.

7. Add 5 µl of Diluent Solution to the “Positive Control" and “Blank” wells.

8. Add 20 µl of 1x Assay Buffer to the "Blank" wells.

9. Thaw **PADI4** on ice. Briefly spin the tube to recover its full content.

10. Dilute the protein (20 µl/well) to 20 ng/µl using 1x Assay Buffer.

    Note: The concentration of protein is lot-specific and is indicated on the tube. Verify the initial concentration and dilute accordingly. This protein is particularly sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use the thawed protein and do not re-use the diluted material.

11. Initiate the reaction by adding 20 µl of diluted **PADI4** to the wells designated “Positive Control” and "Test Inhibitor".

12. Incubate at 37°C for 30 minutes.

13. At the end of the 30-minute reaction, quench the reaction by adding 15 µl of **0.5 M EDTA** to each well.

14. Incubate at Room Temperature (RT) for 3 minutes.

15. Prepare a second plate and add 200 µl of 100 mM Phosphate Buffer, pH 7.8 to each well needed.

16. Transfer 50 µl of the quenched solution from the first plate to the 200 µl of 100 mM Phosphate Buffer, pH 7.8 in the second plate.

17. Prepare 100 mM DTT in ethanol.

18. Mix equal amounts of 100 mM DTT and **100 mM o-phthalaldehyde (OPT)**.

19. Add 10 µl of the DTT/ OPT mix to all wells (final volume in each well is now 260 µl).

20. Incubate for 15 minutes at 37°C.

21. Read sample in a fluorescence plate-reader capable of excitation at a wavelength of 413 nm and detection of emitted light of 476 nm.

22. The "Blank" value should be subtracted from all other values.

Component	**Blank**	**Positive Control**	**Test Inhibitor**
Master Mix	25 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
1x Assay Buffer	20 µl	-	-
Diluted PADI4 (20 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

Example Results

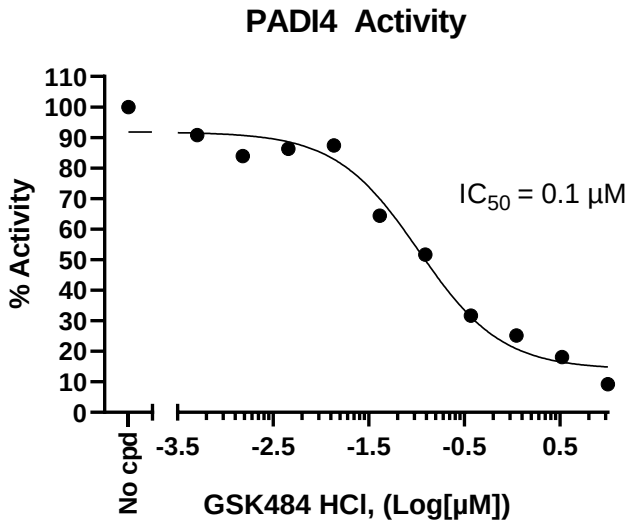

Figure 1: Inhibition of PADI4 activity by GSK484 HCl.
PADI4 activity was measured in the presence of increasing concentrations of GSK484 HCl (SelleckChem #S7803. The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (protein activity in the absence of inhibitor, set at 100%).

Data shown is representative. 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 all further questions, please email support@bpsbioscience.com

References
Wu X., *et al.*, 2023 *Anticancer Agents Med Chem* 23(3): 256-265.

Related Products

Products	*Catalog #*	*Size*
PADI1 Recombinant	50151	10 µg
PADI2, FLAG-Tag Recombinant	50152	10 µg
PADI3, FLAG-Tag Recombinant	50153	10 µg
Anti-PADI4 Polyclonal Antibody	25305	100 µl