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ab273337 Protein Disulfide Isomerase (PDI) Activity Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273337
(use https://www.abcam.cn/ab273337 for china, or
https://www.abcam.co.jp/ab273337 for Japan)

For the determination of Protein Disulfide Isomerase activity in adherent/suspension cells and tissue.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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1. Overview

Protein Disulfide Isomerase (PDI) Activity Assay Kit (Fluorometric) (ab273337) is a simple, sensitive, and high-throughput adaptable kit that utilizes the reductase activity of PDI for determining its activity in a variety of samples.

This kit employs a synthetic quenched fluorescent substrate which gets cleaved in the presence of PDI producing an enhanced fluorometric signal (Ex/Em = 490/580 nm). In the presence of a specific inhibitor, the quenched fluorescent substrate cannot be cleaved by PDI. Detection limit as low as 0.1 mU.

2. Protocol Summary

Prepare all reagents, samples and positive controls.

Prepare standards.

Add all samples to the appropriate wells.

Add Reaction Mix and Background Control Mix to the appropriate wells.

Add Detection Mix to the appropriate wells.

Measure fluorescence in kinetic mode for 20-60 mins at RT.

Determine Protein Disulfide Isomerase activity using equation.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
PDI Activator/PDI Activator (100 mM)	40 µl	-20°C
PDI Assay Buffer II/PDI Assay Buffer	25 ml	-20°C
50X PDI Inhibitor/PDI Inhibitor (50X)	100 µl	-20°C
PDI Positive Control/PDI Positive Control Lyophilized	1 vial	-20°C
PDI Substrate	200 µl	-20°C
Fluorescence Standard VI/Fluorescence Standard (25 µM)	200 µl	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Multi-well spectrophotometer
- 96-well white plate with flat bottom

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

– Briefly centrifuge small vials at low speed prior to opening.

9.1 **Fluorescence Standard VI/Fluorescence Standard (25 µM):**

Before use, thaw at room temperature. Store at -20°C.

9.2 **PDI Activator/PDI Activator (100 mM):**

Before use, thaw at room temperature. Keep on ice while in use. Store at -20°C.

9.3 **PDI Assay Buffer II/PDI Assay Buffer:**

Warm to room temperature before use. Store at -20°C or 4°C.

9.4 **50X PDI Inhibitor/PDI Inhibitor (50X):**

Before use, thaw at room temperature. Store at -20°C.

9.5 **PDI Positive Control/PDI Positive Control Lyophilized:**

Reconstitute with 22 µl PDI Assay Buffer II/PDI Assay Buffer and mix thoroughly. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

9.6 **PDI Substrate:**

Before use, thaw at room temperature. Keep on ice while in use. Store at -20°C.

10. Sample Preparation

Tissue/cell lysate preparation:

10.1 Rapidly homogenize tissue (50 mg) or cells (5-10 x 10^6) with 200 µl ice-cold PDI Assay Buffer II/PDI Assay Buffer and keep samples on ice for 10 mins.

10.2 Centrifuge at 10,000 *x g* for 5 mins at 4°C to remove cell debris and transfer the supernatant to a fresh tube.

10.3 Small molecules in sample may interfere with the assay. Remove small molecules by using ammonium sulfate: pipette 50-100 µl of lysate into a fresh tube; add 2 X volume of saturated ammonium sulfate (4.1 M at RT) and then keep on ice for 20 mins.

10.4 Spin down at 10,000 *x g* for 5 mins. at 4°C, carefully remove and discard the supernatant, and resuspend the pellet to the original volume with ice-cold PDI Assay Buffer II/PDI Assay Buffer.

10.5 Add 5-50 µl of reconstituted sample in two parallel wells (Sample and Sample Background Control) and adjust their volume to 50 µl with PDI Assay Buffer II/PDI Assay Buffer.

10.6 For PDI Positive Control. Add 1-5 µl of PDI Positive Control into desired well(s) and adjust the volume to 50 µl/well with PDI Assay Buffer II/PDI Assay Buffer.

Δ Note: For unknown samples, we suggest testing several doses of your sample to make sure the readings are within the standard curve range.

Δ Note: Do not store the diluted PDI Positive Control.

11. Standard Curve

– Keep standards on ice while in use.

11.1 Add 0, 2, 4, 6, 8 and 10 µl of 25 µM Fluorescence Standard VI/Fluorescence Standard into a series of wells in a 96-well white plate to generate 0, 50, 100, 150, 200 and 250 pmol/well of Fluorescence Standard.

11.2 Adjust the volume to 100 µl/well with PDI Assay Buffer II/PDI Assay Buffer.

Standard #	25 µM Fluorescence Standard VI/Fluorescence Standard (µL)	PDI Assay Buffer II/PDI Assay Buffer (µL)	Fluorescence Standard (pmol/well)
1	0	100	0
2	2	98	50
3	4	96	100
4	6	94	150
5	8	92	200
6	10	90	250

12. Assay Procedure

– Keep on ice while in use.

12.1 **Reaction Mix:**
Dilute 100 mM PDI Activator to 1 mM by adding 10 µl of 100 mM PDI Activator into 990 µl dH2O. Mix well. Prepare enough reaction mix for the number of assays to be performed. For each well, prepare 25 µl Reaction Mix containing:

	Reaction mix (µl)	Background Control mix (µl)
PDI Assay Buffer II/PDI Assay Buffer	24	22
PDI Activator (1 mM)	1	1
50X PDI Inhibitor/PDI Inhibitor (50X)	---	2

12.2 Mix well by vortexing.
12.3 Add 25 µl of Reaction Mix to each well containing Positive Control and samples.
12.4 Add 25 µl of Background Control mix to sample background control well(s).
12.5 Mix well and incubate at room temperature for 15 mins.

Δ Note: Do not store Diluted PDI Activator. Always prepare fresh diluted stock solution.

12.6 **Detection Mix:**
Mix enough reagents for the number of assays to be performed. For each well, prepare 25 µl Detection Mix containing:

	Detection mix (µl)
PDI Assay Buffer II/PDI Assay Buffer	23

PDI Substrate	2

12.7 Mix well by vortexing.
12.8 Add 25 µl of Detection Mix to each well containing Positive Control and samples.
12.9 Measure Fluorescence (Ex/Em = 490/580 nm) immediately in kinetic mode for 20-60 mins at room temperature. We recommend measuring RFU in kinetic mode and choosing two time points (t1 and t2) in the linear range to calculate the PDI activity of the samples. The Fluorescence Standard Curve can be read in Endpoint mode (i.e., at the end of incubation time).

Δ Note: Incubation time depends on the PDI activity in samples. Longer incubation time may be required for samples having low PDI activity.

13. Calculations

13.1 Subtract 0 Standard reading from all readings.
13.2 Plot the Fluorescence Standard Curve.
13.3 If sample background control reading is significant, subtract sample background control reading from sample reading(s).
13.4 Apply corrected RFU to the Fluorescence Standard Curve to get B pmol of Fluorescence generated by PDI during the incubation time (Δt).

$$PDI\ activity\ = \frac{B}{(2\ x\ \Delta t\ x\ V)} X\ D = pmol/min/\mu l = \mu U/\mu l$$

B = Fluorescence amount in the sample well from Standard Curve (pmol)
Δt = Reaction time ($t_2 - t_1$) (mins)
2 = Conversion Factor: PDI converts 1 pmol of Substrate, generating 2 pmoles of Fluorophore
V = Sample volume added into the reaction well (µl).

Unit Definition: One unit of PDI is the amount of enzyme that generates 1 µmol Fluorescence at Ex/Em = 490/580 nm per minute in

the presence of PDI Activator at pH 7.5 at 25°C.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

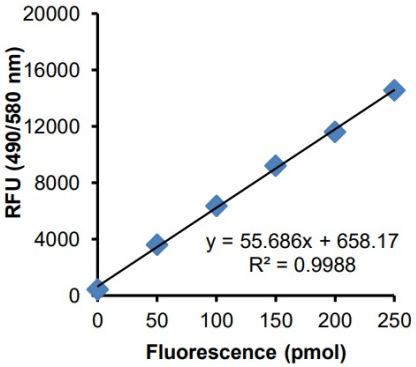

Figure 1. Fluorescence Standard Curve.

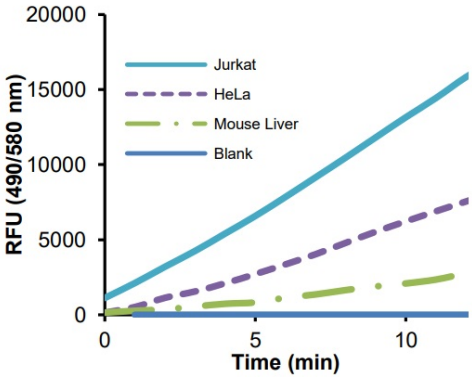

Figure 2. Measurement of PDI activity in mouse liver lysate, Jurkat and HeLa cell extract.

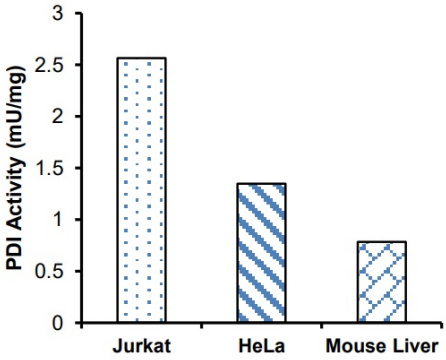

Figure 3. Relative PDI Activity was calculated in Jurkat cell extract (9.2 µg), HeLa cell extract (9.1 µg) and liver lysates prepared from mice liver (4.6 µg).

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)