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

9/16

(Catalog # K949-100; 100 assays; Store at -20°C)

I. Introduction:

Protein Disulfide Isomerases (PDI, EC: 5.3.4.1) are multifunctional proteins that constitute a thio-disulfide oxidoreductase family. PDI is abundant in the lumen of endoplasmic reticulum (ER). PDI plays an essential role in catalyzing the rearrangement of disulfide (S-S) bonds in proteins and functions as a chaperone by inhibiting protein aggregation. Recent studies show that PDI activity is essential for cancer cell survival and proliferation, and targeting PDI activity with specific inhibitors abrogates survival responses leading to ER stress in cancer cells. Therefore, the detection of PDI activity is critical for mechanistic study, diagnosis, prevention, and therapeutic purposes. BioVision's PDI Activity Assay kit utilizes the reductase activity PDI for determining its activity in a variety of samples. The 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 BioVision's PDI specific inhibitor, the quenched fluorescent substrate cannot be cleaved by PDI. BioVision's PDI Activity Assay Kit is simple, sensitive, and high-throughput adaptable with the detection limit as low as 0.1 mU.

Quenched Substrate $\xrightarrow{\text{PDI + PDI Activator}}$ Reduced Substrate (Ex/Em=490/480 nm)

II. Application:

- Measurement of PDI activity in various biological samples
- Study/characterize of potential PDI inhibitors

III. Sample Type:

- Animal tissues such as liver, kidney, etc.
- Adherent or suspension cells

IV. Kit Contents:

Components	K949-100	Cap Code	Part Number
PDI Assay Buffer	25 ml	WM	K949-100-1
PDI Substrate	200 µl	Red	K949-100-2
PDI Activator (100 mM)	40 µl	Blue	K949-100-3
PDI Inhibitor (50X)	100 µl	Brown	K949-100-4
PDI Positive Control	1 vial	Orange	K949-100-5
Fluorescence Standard (25 µM)	200 µl	Yellow	K949-100-6

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **PDI Assay Buffer:** Warm to room temperature before use. Store at -20°C or 4°C.
- **PDI Substrate and PDI Activator (100 mM):** Before use, thaw at room temperature. Keep on ice while in use. Store at -20°C.
- **PDI Inhibitor (50X) and Fluorescence Standard (25 µM):** Before use, thaw at room temperature. Store at -20°C.
- **PDI Positive Control:** Reconstitute with 22 µl PDI Assay Buffer and mix thoroughly. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

VII. PDI Activity Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (50 mg) or cells (5-10 x 10^6) with 200 µl ice-cold PDI Assay Buffer, and keep samples on ice for 10 min. Centrifuge at 10,000 x g for 5 min at 4°C to remove cell debris, and transfer the supernatant to a fresh tube. 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 [Cat. # 7096] at room temperature) and then keep on ice for 20 min. Spin down at 10,000 x g for 5 min. at 4°C, carefully remove and discard the supernatant, and resuspend the pellet to the original volume with ice-cold PDI Assay Buffer. Add 5-50 µl of reconstituted sample in two parallel wells (Sample & Sample Background Control) and adjust their volume to 50 µl with PDI Assay Buffer. 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.

   Notes:

   a. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.
   b. Do not store the diluted PDI Positive Control.

2. **Standard Curve:** Add 0, 2, 4, 6, 8 and 10 µl of 25 µM 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. Adjust the volume to 100 µl/well with PDI Activity Assay Buffer.
3. **Reaction Mix:** Dilute 100 mM PDI Activator to 1 mM by adding 10 µl of 100 mM PDI Activator into 990 µl dH_2O. 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	Background Control Mix*
PDI Assay Buffer	24 µl	22 µl
PDI Activator (1 mM)	1 µl	1 µl
PDI Inhibitor (50X)	---	2 µl

Mix well by vortexing. Add 25 µl of Reaction Mix to each well containing Positive Control and samples. Mix well and incubate at room temperature for 15 min.

Notes:

a. Do not store Diluted PDI Activator. Always prepare fresh diluted stock solution.
b. *Add 25 µl of Background Control Mix to sample background control well(s).

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

PDI Assay Buffer	23 µl
PDI Substrate	2 µl

Mix well by vortexing. Add 25 µl of Detection Mix to each well containing Positive Control and samples.

5. **Measurement:** Measure Fluorescence (Ex/Em = 490/580 nm) immediately in kinetic mode for 20-60 min. at room temperature. We recommend measuring RFU in kinetic mode, and choosing two time points (t1 & 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).

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

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

Sample PDI Activity = B/(2 X Δt X V) x Dilution Factor = pmol/min/µl = µU/µl = mU/ml

Where: **B** is Fluorescence amount in the sample well from Standard Curve (pmol).
2 is the Conversion Factor: PDI converts 1 pmol of Substrate, generating 2 pmoles of Fluorophore.
Δt is reaction time (min.), $\Delta t = t_2 - t_1$
V is 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.

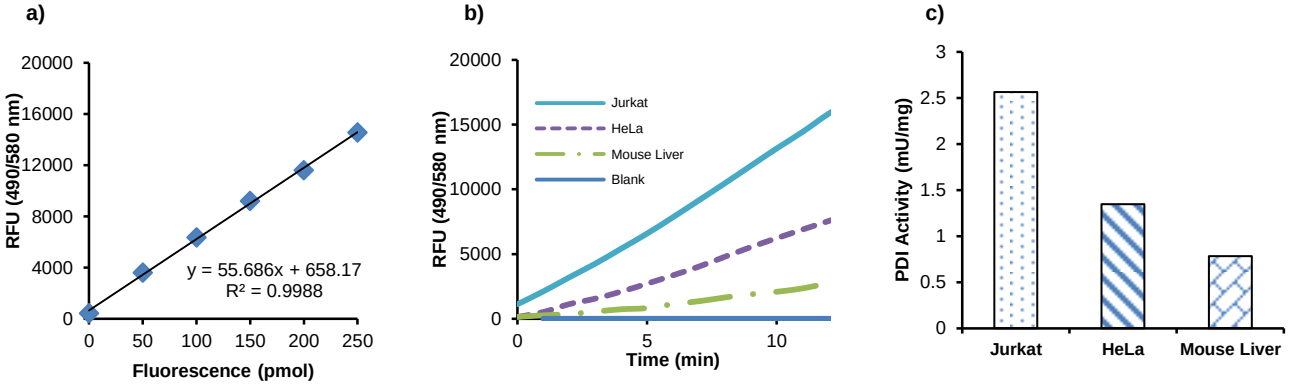

Figure: (a) Fluorescence Standard Curve. (b) Measurement of PDI activity in Mouse Liver lysate, Cell Extracts from Jurkat Cells and HeLa Cells. (c) Relative PDI Activity was calculated in Jurkat Cell Extract (9.2 µg), HeLa Cell Extract (9.1 µg) and liver lysates prepared from mice liver (4.6 µg). Assay was performed following the kit protocol.

VIII. RELATED PRODUCTS:

PDI Inhibitor Screening Assay Kit (K840)
Lipid Peroxidation (MDA) Colorimetric/Fluorometric Assay Kit (K739)
Myeloperoxidase (MPO) Colorimetric Assay Kit (K744)
Myeloperoxidase Fluorometric Assay Kit (K745)
MPO Inhibitor Screening Kit (K746)
MPO Peroxidation Assay Kit (K747)
Sphingomyelinase Activity Colorimetric Assay Kit (K599)
Sphingomyelinase Activity Fluorometric Assay Kit (K574)
Sphingomyelin Quantification Colorimetric Assay Kit (K600)

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