

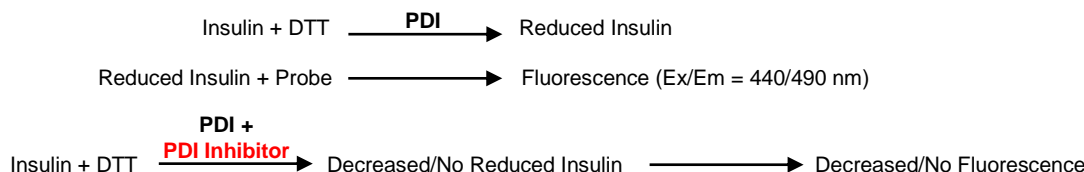
## Protein Disulfide Isomerases (PDI) Inhibitor Screening Kit (Fluorometric)

6/15

**(Catalog # K840-100; 100 assays; Store at -20°C)**

## I. Introduction:

Protein Disulfide Isomerases (PDI, EC: 5.3.4.1) 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 S-S bonds in proteins and functions as a chaperon. Recent studies show that PDI activity is essential for cancer cell survival and proliferation, and targeting the PDI activity with its inhibitors abrogates survival responses to ER stress in cancer cells. Thus, identification and development of PDI inhibitors represents an important approach in cancer therapy. In BioVision's PDI Inhibitor Screening Kit, the PDI converts Insulin into its reduced form in the presence of Dithiothreitol (DTT). This reduced insulin binds to a fluorescent probe to generate an intensely fluorescent product (Ex/Em = 440/490 nm). In the presence of a PDI inhibitor, the reaction is impeded/abolished, resulting in a decrease or total loss of fluorescence. This assay kit can be used to screen/study/characterize the potential inhibitors of Protein Disulfide Isomerase. The assay is simple, high-throughput adaptable and can be performed within 60 min.



## II. Application:

- Screening/characterizing/studying potential inhibitors of Protein Disulfide Isomerase.

### III. Kit Contents:

Components	K840-100	Cap Color	Part Number
PDI Assay Buffer	25 ml	WM	K840-100-1
PDI Substrate	2 vials	Orange	K840-100-2
PDI Probe (in DMSO) (20X)	20 µl	Red	K840-100-3
DTT (100X)	100 µl	Green	K840-100-4
PDI Enzyme	1 vial	Yellow	K840-100-5
PDI Inhibitor Control (Iodoacetamide)	1 vial	Brown	K840-100-6

**IV. User Supplied Reagents and Equipment:**

- 96-well white plate with flat bottom
- Multi-well spectrophotometer (fluorescent plate reader)
- 10 mM HCl solution

#### V. 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:** Bring to room temperature before use. Store at -20°C or 4°C.
- **PDI Substrate:** Reconstitute one vial of PDI Substrate with 1.1 ml of 10 mM HCl solution (not provided). Aliquot and store at -20°C. Avoid freeze/thaw. Keep on ice while in use. Use within two months.
- **PDI Enzyme:** Reconstitute with 550 µl PDI Assay Buffer. Aliquot and store at -20°C. Avoid freeze/thaw. Keep on ice while in use. Use within two months.
- **PDI Inhibitor Control:** Reconstitute with 1 ml PDI Assay Buffer. Aliquot and store at -20°C. Avoid freeze/thaw. Keep on ice while in use. Use within two months.

## VI. PDI Inhibitor Screening Protocol:

**1. Screen Compounds, Inhibitor Control, and Enzyme Control Preparation:** Dissolve candidate inhibitors into an appropriate solvent to make the stock solution. Dilute to 2X desired test concentration with the PDI Assay Buffer. Add 50 µl diluted candidate inhibitor or PDI Assay Buffer into desired wells, as Sample [S], or Enzyme Control [EC] (no inhibitor). For Inhibitor Control (IC), dilute Inhibitor Control 10 times by adding 10 µl Inhibitor Control to 90 µl PDI Assay Buffer. Add 50 µl of diluted Inhibitor Control into desired well(s).

**Note:** Solvents used to solubilize the inhibitors might affect the enzymatic activity. If solvent effect on the enzymatic activity is a concern, prepare a solvent control well(s) (SC) with the same final concentration of the solvent(s) as in the inhibitor sample(s).

**2. PDI Enzyme:** Add 5 µl of PDI Enzyme into Sample, Enzyme Control, and Inhibitor Control wells (if necessary, in Solvent Control wells). Add 55 µl of Assay Buffer into separate well designated as BC (Background Control). Incubate for 30 min. at 37°C.

**3. Substrate Solution Preparation:** Prepare 1X DTT by taking 10 µl of 100X DTT into 990 µl PDI Assay Buffer and Mix well.\* Dilute the 20X PDI probe to 1X by adding 10 µl of PDI probe into 190 µl PDI Assay Buffer and Mix well.\*\* Make enough reagents for the number of assays to be performed. For each well, prepare 45 µl of Substrate solution containing:

PDI Assay Buffer	21 $\mu$ l
PDI Substrate	20 $\mu$ l

DTT (1X) 2 µl  
PDI Probe (1X) 2 µl

Mix and add 45 µl of Substrate solution into each well from step VI. 2. Mix well with gentle shaking, protected from light and incubate for 10 min at 37°C.

**Note:** \* & \*\* Make fresh dilutions each time. Do not store the diluted DTT and diluted PDI Probe.

**4. Measurement:** Measure fluorescence (Ex/Em = 440/490 nm) in kinetic mode for 5-30 min. at 37°C. Choose two time points (T<sub>1</sub> & T<sub>2</sub>) in the linear range of the enzyme kinetics and obtain the corresponding values for the fluorescence (RFU<sub>1</sub> & RFU<sub>2</sub>).

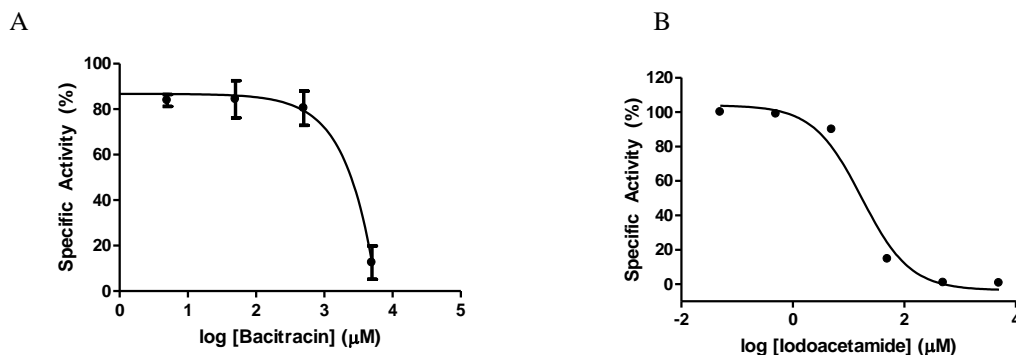
**5. Calculations:** Subtract the background from all samples (ΔBC = BC<sub>2</sub>-BC<sub>1</sub>). Calculate the slope for all Samples (S), including Enzyme Control (EC), by dividing the corrected ΔRFU (RFU<sub>2</sub>-RFU<sub>1</sub>) values with the time ΔT (T<sub>2</sub>-T<sub>1</sub>).

$$\% \text{ Inhibitor} = \frac{(\text{Slope of EC} - \text{Slope of S})}{\text{Slope of EC}} \times 100$$

$$\% \text{ Specific Activity} = \frac{\text{Slope of S}}{\text{Slope of EC}} \times 100$$

**Notes:**

- Irreversible inhibitors that inhibit the PDI activity completely at the tested concentration will have ΔRFU = 0 and thus the % Inhibition will be 100%.
- In case Solvent Control(s) has substantially different slope(s) than the EC, use SC slope(s) instead of Slope of EC in the equation above.



**Figure:** Inhibition of Protein Disulfide Isomerase activity by PDI Inhibitors (Bacitracin and Iodoacetamide). For Iodoacetamide, IC<sub>50</sub> = 17 µM. Assay was performed following the kit protocol.

**VII. Related Products:**

Beta-Galactosidase Staining Kit (K802)  
Factor Xa Inhibitor Screening Kit (K362)  
Myeloperoxidase (MPO) Inhibitor Screening Kit (K746)  
pCAF Inhibitor Screening Assay (K345)  
HMG-CoA Reductase Activity/Inhibitor Screening kit (K588)  
SIRT2 Inhibitor Screening Assay Kit (K322)  
Beta-Galactosidase Activity Assay Kit (K821-100)

AHCY Inhibitor Screening Kit (K326)  
Human Calpain 1 Inhibitor Screening Kit (K244)  
p300 Inhibitor Screening Kit (K346)  
TACE Inhibitor Screening Assay Kit (K366)  
SIRT1 Inhibitor/Activator Screening Kit (K325)  
SIRT6 Inhibitor Screening Assay Kit (K323)  
Protein Disulfide Isomerase (PDI), human recombinant (7601)

**FOR RESEARCH USE ONLY! Not to be used on humans.**