ab284515 – Protein Disulfide Isomerases (PDI) Inhibitor Screening Kit (Fluorometric)

For the screening of potential PDI inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284515

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
PDI Assay Buffer	25 mL	-20°C
PDI Substrate	2 x vials	-20°C
PDI Probe (in DMSO) (20X)	20 µl	-20°C
DTT (100X)	100 µl	-20°C
PDI Enzyme	1 x vial	-20°C
PDI Inhibitor Control (Iodoacetamide)	1 x vial	-20°C

Materials Required, Not Supplied

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

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

Reagent Preparation

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening.

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.

Assay Protocol

Screen Compounds, Inhibitor Control, and Enzyme Control Preparation:

1. Dissolve candidate inhibitors into an appropriate solvent to make the stock solution.
2. Dilute to 2X desired test concentration with the PDI Assay Buffer.
3. Add 50 µl diluted candidate inhibitor or PDI Assay Buffer into desired wells, as Sample [S], or Enzyme Control [EC] (no inhibitor).
4. 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).

Reaction Mix:

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

Substrate Mix:

1. 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:

	Substrate Mix
PDI Assay Buffe	21 µl
PDI Substrate	20 µl
DTT (1X)	2 µl
PDI Probe (1X)	2 µl

2. 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.

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

Measurement

Measure fluorescence (Ex/Em = 440/490 nm) in kinetic mode for 5-30 min. at 37°C. Choose two time points (T1 & T2) in the linear range of the enzyme kinetics and obtain the corresponding values for the fluorescence (RFU1 & RFU2).

Calculation:

1. Subtract the background from all samples (ΔBC = BC2-BC1).
2. Calculate the slope for all Samples (S), including Enzyme Control (EC), by dividing the corrected ΔRFU (RFU2-RFU1) values with the time ΔT (T2-T1).

$$\% \text{ Inhibition} = \frac{slope\ of\ EC - slope\ of\ S}{slope\ of\ EC} \times 100$$

$$\% \text{ Specific activity} = \frac{slope\ of\ S}{slope\ of\ EC} \times 100$$

Technical Support

4. For all technical or commercial enquiries please go to:
5.
6. www.abcam.com/contactus
7. www.abcam.cn/contactus (China)

1. Version 1, Last updated Thursday, August 5, 2021

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

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