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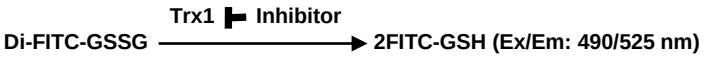
Human Thioredoxin 1 (Trx1) Inhibitor Screening Kit (Fluorometric)

8/17

(Catalog # K535-100; 100 assays; Store at -20°C)

I. Introduction:

The human Thioredoxin 1 (Trx1) is a key enzyme that protects cells from oxidative stress by reducing disulfides and methionine sulfoxide groups in oxidized proteins. It also regulates DNA repair and apoptosis. In mammals, Trx1 is necessary for growth and development. However, an aberrant elevated expression of Trx1 is known to promote lung, cervical, pancreatic, colorectal, and breast cancers. In BioVision's Trx1 Inhibitor Screening Kit, Trx1 reduces quenched di-FITC-labeled oxidized glutathione (Trx1 substrate), to mono-FITC-labeled unquenched reduced glutathione. In the presence of a Trx1 inhibitor (control inhibitor included), the reaction is impeded resulting in lower or no signal. The assay is high-throughput adaptable and can be completed in less than 1 hr.



II. Application:

- Screening/characterizing/studying potential inhibitors for Trx1.

III. Kit Contents:

Components	K535-100	Cap Code	Part Number
Trx1 Assay Buffer	25 ml	WM	K535-100-1
Trx1 Substrate (Lyophilized)	1 vial	Brown	K535-100-2
DTT (1 M)	100 µl	Green	K535-100-3
Trx1 Enzyme (Lyophilized)	1 vial	Blue	K535-100-4
Trx1 Inhibitor (50X)	35 µl	Clear	K535-100-5

IV. User Supplied Reagents and Equipment:

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

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Warm Trx1 Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **Trx1 Enzyme:** Reconstitute vial with 220 µl of ddH_2O. Aliquot and store at -20°C.
- **Trx1 Substrate Mix:** Dissolve with 660 µl of Trx Assay Buffer. Aliquot and store at -20°C protected from light.

VI. Trx Inhibitor Screening Protocol:

1. **Screen Compounds, Inhibitor Control, and Enzyme Control Preparation:** Dissolve candidate inhibitors into an appropriate solvent at highest concentration to be tested. Dilute to 2X desired test concentration with Trx1 Assay Buffer. Add 50 µl diluted candidate inhibitor or Trx1 Assay Buffer into desired wells for Sample Inhibitors [S], and Enzyme Control [EC] (no inhibitor) respectively. For the Inhibitor Control (IC), add 2 µl of Inhibitor Control and adjust the volume to 50 µl with Trx1 Assay Buffer.

   Note: Solvents used to solubilize the inhibitors might affect the enzymatic activity. Prepare a solvent control [SC] to test the effect of solvent on the enzymatic activity.

2. **DTT:** Dilute 1 M DTT to 1 mM. For example, taking 2 µl of 1 M DTT and adding it to 2 ml of ddH_2O.

3. **Enzyme Solution:** Mix enough reagents for the number of assays to be performed. For each well, prepare 20 µl of Trx1 Enzyme Solution in the following manner:

Trx1 Assay Buffer	16 µl
Trx1 Enzyme	2 µl
1 mM DTT	2 µl

   Add Enzyme Solution to the wells containing Sample Inhibitors, Enzyme Control, Inhibitor Control, and Solvent Control. Incubate the mixture for 15 minutes at 25°C.

4. **Substrate**: Dilute (5-fold) substrate for use. For example, 100 µl of substrate can be diluted in 400 µl of Trx1 Assay Buffer. To each well, add 30 µl of the diluted Trx1 Substrate. Mix well with gentle shaking.

5. **Measurement:** Measure fluorescence at Ex/Em = 490/525 nm in kinetic mode for 15 min at 25°C. Choose two time points (t_1 & t_2) in the linear range of the curve and obtain the corresponding values for the $RFU_{490/525nm}$ (RFU_1 & RFU_2).

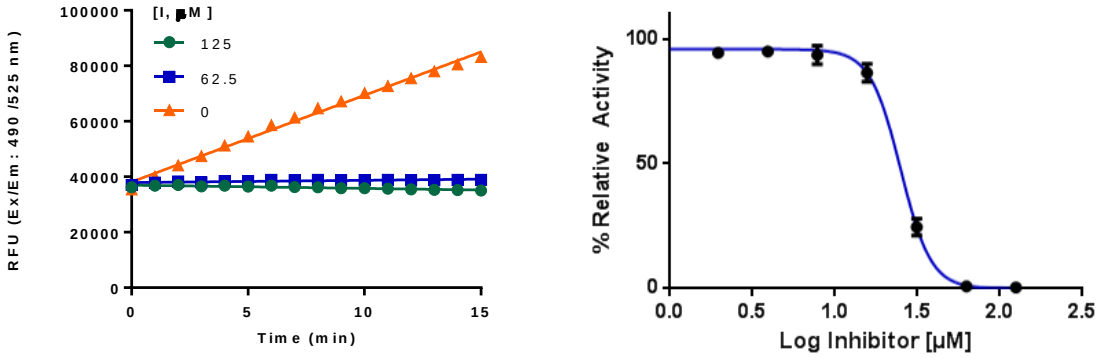
6. **Calculations:** Calculate the slope for all sample including the Enzyme Control (EC), Inhibitor Control (IC), Sample (S), and Solvent Control. Consider the EC as 100%, by dividing the net $\Delta RFU = (RFU_2 - RFU_1)$ value by the time $\Delta t = (t_2 - t_1)$. Calculate % relative inhibition as follows:

   Note: If the activity measured in your solvent control (SC) is significantly different from that in your enzyme control (EC) use the SC value instead of EC value in the equations below:

$$\textbf{Relative Activity}\ (\%) = \frac{\textbf{Slope of S}}{\textbf{Slope of EC}}\ \textbf{X 100}$$

$$\textbf{Relative Inhibition}\ (\%) = \frac{\textbf{Slope of EC} - \textbf{Slope of S}}{\textbf{Slope of EC}}\ \textbf{X 100}$$

Where: **Slope of EC** is the slope of Enzyme Control
Slope of S is the slope of Sample Screen



Figures: A) Effects of 125 µM, 62.5 µM, and 0 µM of Trx1 inhibitor on Trx1 activity. **B)** Inhibition of Trx1 activity by Trx1 Inhibitor (IC_{50} = 25 µM). Assays were performed following the kit protocol.

VII. RELATED PRODUCTS:

Thioredoxin Reductase Activity Colorimetric Assay Kit (K763)
Protein Disulfide Isomerases (PDI) Inhibitor Screening Kit (Fluorometric)(Colorimetric) (K840)
Thioredoxin isoform 1 (Trx), Active, Human Recombinant (P1039)
Thioredoxin 1 Antibody (3A1) (6166)
PDI (Protein Disulfide Isomerase) Antibody (5601)
Protein Disulfide Isomerase (PDI), human recombinant (7601)
Thioredoxin 1 (Trx) Fluorometric Substrate, Di- FITC oxidized glutathione (FGSSGF) (9699)

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