Thioredoxin Reductase Microplate Assay Kit User Manual

Catalog # FTA0041

(Version 1.1C)

Detection and Quantification of Thioredoxin Reductase (TrxR) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Thioredoxin reductase (TrxR) (EC 1.8.1.9) is a ubiquitous enzyme which is involved in many cellular processes such as cell growth, p53 activity, and protection against oxidation stress, etc. The mammalian TrxR reduces thioredoxins as well as non-disulfide substrates such as selenite, lipoic acids, lipid hydroperoxides, and hydrogen peroxide.

Thioredoxin Reductase Microplate Assay Kit provides a convenient colorimetric assay for detecting TrxR activity in various samples. In the assay TrxR catalyzes the reduction of 5, 5'-dithiobis (2-nitrobenzoic) acid (DTNB) with NADPH to 5-thio-2-nitrobenzoic acid (TNB2-), which generate a strong yellow color (λmax = 412 nm).

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Inhibitor	1 ml x 1	4 °C, keep in dark
Substrate	Powder x 1	-20 °C, keep in dark
Dye Reagent	Powder x 1	-20 °C, keep in dark
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Note:

Substrate: add 4 ml Assay Buffer to dissolve before use.

Dye Reagent: add 20 ml distilled water to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 412 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 8,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Warm the Assay Buffer to room temperature before use.

Add following reagents in the microplate:

Reagent	Sample	Control
Sample	20 µl	20 µl
Assay Buffer	60 µl	50 µl
Inhibitor	--	10 µl
Substrate	20 µl	20 µl
Dye Reagent	100 µl	100 µl
Mix, measured at 412 nm and record the absorbance of 10th second and 130th second.		

VI. CALCULATION

Unit Definition: One unit of TrxR activity is the enzyme that reduces 1 nmol of DTNB per minute.

1. According to the protein concentration of sample

$$TrxR\ (U/mg) = [(OD_{Sample\ (130S)} - OD_{Control\ (130S)}) - (OD_{Sample\ (10S)} - OD_{Control\ (10S)})] / (\varepsilon \times d) \times V_{Total} / (V_{Sample} \times C_{Protein}) / T$$

$$= 612.7 \times [(OD_{Sample\ (130S)} - OD_{Control\ (130S)}) - (OD_{Sample\ (10S)} - OD_{Control\ (10S)})] / C_{Protein}$$

2. According to the weight of sample

$$TrxR\ (U/g) = [(OD_{Sample\ (130S)} - OD_{Control\ (130S)}) - (OD_{Sample\ (10S)} - OD_{Control\ (10S)})] / (\varepsilon \times d) \times V_{Total} / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 612.7 \times [(OD_{Sample\ (130S)} - OD_{Control\ (130S)}) - (OD_{Sample\ (10S)} - OD_{Control\ (10S)})] / W$$

3. According to the quantity of cells or bacteria

$$TrxR\ (U/10^4) = [(OD_{Sample\ (130S)} - OD_{Control\ (130S)}) - (OD_{Sample\ (10S)} - OD_{Control\ (10S)})] / (\varepsilon \times d) \times V_{Total} / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 612.7 \times [(OD_{Sample\ (130S)} - OD_{Control\ (130S)}) - (OD_{Sample\ (10S)} - OD_{Control\ (10S)})] / N$$

ε: molar extinction coefficient, 13.6×10^{-3} L/μmol/cm = 13.6×10^{-3} ml/nmol/cm;

d: the optical path of 96-Well microplate, 0.6 cm;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

V_{Total}: the total volume of the enzymatic reaction, 0.2 ml;

V_{Sample}: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 2 minutes.

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

VIII. NOTES