TBARS Microplate Assay Kit

Cat #: orb545625 (manual)

Detection and Quantification of Thiobarbituric Acid Reactive Substances (TBARS) Content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluids Samples.

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

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INTRODUCTION

Oxidative attack of essential cell components by reactive oxygen species has been associated with several human diseases, such as atherosclerosis, cardiovascular diseases, diabetes, liver disorders, and inflammatory rheumatic diseases. Thiobarbituric Acid Reactive Substances (TBARS) are low-molecular-weight end products (mainly malondialdehyde, MDA) that are formed during the decomposition of lipid peroxidation products. Increased levels of TBARS have been demonstrated in these diseases

TBARS Microplate Assay Kit is a sensitive assay for determining TBARS concentration in various samples. TBARS concentration is based on the reaction of TBARS with thiobarbituric acid (TBA) to form a pink colored product. The color intensity at 535 nm is directly proportional to TBARS concentration in the sample.

KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	10 ml x 1	RT
Dye Reagent	15 ml x 1	RT
Standard (25 µmol/L)	1 ml x 1	RT
Technical Manual	1 Manual	

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 535 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. PBS

SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml PBS for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times) ; centrifuged at 8000g 4 °C for 10 minutes. Transfer 100 µl sample into a micro-centrifuge tube. Then add 100 µl Assay buffer into a micro-centrifuge tube. Incubate for 5 minutes on ice; centrifuged at 12000g 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 PBS on ice, centrifuged at 8000g 4 °C for 10 minutes. Transfer 100 µl sample into a micro-centrifuge tube. Then add 100 µl Assay buffer into a micro-centrifuge tube. Incubate for 5 minutes on ice; centrifuged at 12000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum, plasma or other biological fluids samples

Transfer 100 µl sample into a micro-centrifuge tube. Then add 100 µl Assay buffer into a micro-centrifuge tube. Incubate for 5 minutes on ice; centrifuged at 12000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

ASSAY PROCEDURE

Warm the Dye Reagent to 37 °C before use.

Add following reagents in the microcentrifuge tube:

Reagent	Sample	Standard	Blank
Sample	150 µl	--	--
Standard	--	150 µl	--
Distilled water	--	--	150 µl
Dye Reagent	150 µl	150 µl	150 µl
Shake and mix, put them into boiling water bath for 10 minutes. When cold, add the Supernatant into the microplate.			
Supernatant	200 µl	200 µl	200 µl
Record absorbance measured at 535 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

CALCULATION

1. According to the protein concentration of sample

$$TBARS\ (\mu mol/mg) = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) \times 2$$

$$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$TBARS\ (\mu mol/g) = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{PBS}) \times 2$$

$$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the volume of sample

$$TBARS\ (\mu mol/ml) = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} \times 2$$

$$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

4. According to the quantity of cell or bacteria

$$TBARS\ (\mu mol/10^4\ cell) = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{PBS}) \times 2$$

$$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Standard}$: the standard concentration, 25 μmol/L = 0.025 μmol/ml;

$V_{Standard}$: the volume of standard, 0.15 ml;

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

W: the weight of sample, g;

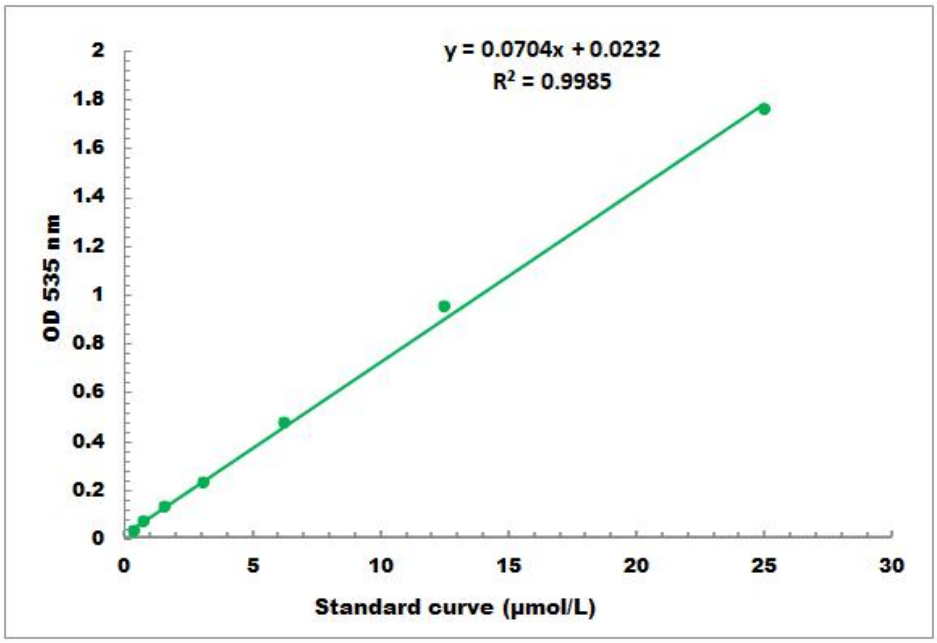
V_{Sample}: the volume of sample, 0.15 ml;

V_{PBS}: the volume of PBS, 1 ml;

N: the quantity of cell or bacteria, $N \times 10^4$.

TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.5 µmol/L - 25 µmol/L

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