Thiobarbituric Acid Reactants (TBARS) Colorimetric Assay Kit

Catalog Number EEA021 (96 tests)

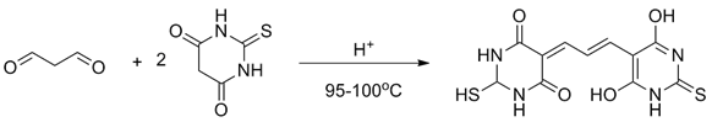
Rev 2.0

For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

This kit can be used to measure thiobarbituric acid reactive substances (TBARS) concentration in serum, plasma, animal tissue, culture cells, and other samples. The organism produces oxygen free radicals through the enzyme and non-enzyme systems, attacks polyunsaturated fatty acids in biofilm, induces lipid peroxidation, and thus forms lipid peroxides. Malondialdehyde (MDA) is one of the common products of lipid peroxidation in organisms. In clinical science, MDA is a biomarker of lipid peroxidation, which can reflect the degree of lipid peroxidation in organism and indirectly reflect the degree of cell injury.

TBARS and thiobarbituric acid (TBA) can react under high temperature and acidic conditions to form a pink compound where the concentration of which is linearly related to the concentration of TBARS in the sample. The TBARS concentration can be calculated by measuring the OD values at 530-540 nm.



Contents and storage

Kit and components are shipped at 2-8℃. An unopened kit can be stored at 2-8℃ for 12 months.

Components	Quantity (96 tests)
Clarificant	12 mL
Acid Reagent	12 mL
TBA Reagent	Powder
200 μmol/L Standard	5 mL
Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water
- Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)
- Glacial acetic acid
- Microtiter plate reader with software capable of measurement at or near 532 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions
- Incubator capable of maintaining 37℃ and 100℃

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Sample requirements

- Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their experiments.
- If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
- If a lysis buffer is used to prepare tissue or cell homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.

Serum and plasma samples: Detect the sample directly. If the sample is turbid, centrifuge at 10000 g for 10 min at 4℃, then take the supernatant for detection.

Tissue sample:

- Take 0.02-1 g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ to remove blood cells.
- Absorb the water with filter paper and weigh.
- Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) (2-8℃) (mL): the weight of the tissue (g) = 9:1. then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃.
- Take the supernatant and preserve it on ice for detection.

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227).

If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for 1 month.

Prepare samples

It is recommended to take 2~3 samples with expected large difference to do a pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (2.6-100μmol/L).

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Human plasma	1
Rat serum	1
Mouse plasma	1
10% Mouse brain tissue homogenization	1
10% Rat liver tissue homogenization	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)

Preparation of Clarificant solution

The clarificant solution will solidify when stored at 2-8℃. Incubate the vial at 37℃ until fully liquidated.

Preparation of Acid reagent application solution

Mix 1.2 mL of acid reagent and 34 mL of distilled water fully. Prepare the fresh solution before use, where it can be stored at 2-8℃ for one day.

Preparation of TBA reagent application solution

Dissolve a vial of TBA reagent powder with 60 mL of distilled water (90-100℃) and mix fully. Then add 60 mL of glacial acetic acid (self-prepared), mix fully and cool to room temperature. The prepared TBA reagent application solution can be stored at 2~8℃ in the dark for 1 month.

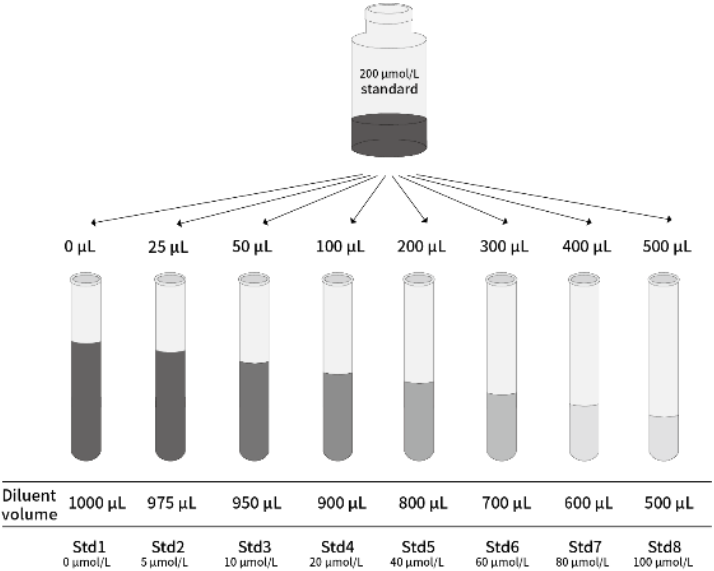
Preparation of chromogenic agent

Fully mix 3 parts of Acid reagent application solution with 1 part of TBA reagent application solution. Prepare the fresh solution before use, where it must be used within 24 hours.

Prepare diluted standards

Note: Use glass or plastic tubes for diluting standards.

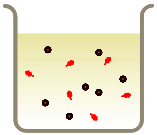
Dilute 200 μmol/L Standard with distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 20, 40, 60, 80, 100 μmol/L.



The key point of the assay

- The temperature of water-bath and the time of incubation should be stabilized (95-100℃, 60 min).
- In the incubation of 100℃ water bath, the EP tube should not be closed directly. It is recommended to fasten the tube mouth with fresh-keeping film and make a small hole in the film.
- The supernatant for colorimetric measurement should not contain sediment, otherwise it will affect the OD values. It is recommended to use a pipette to take the supernatant.

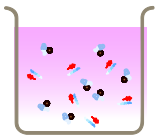
Assay Protocol



1. Add sample and standard

a. **Standard tube:** Take 0.1 mL of standard solution with different concentrations into numbered 10 mL glass tubes.

b. **Sample tube:** Take 0.1 mL of sample into numbered 10 mL glass tubes.



2. Add substrate

a. Add 0.1 mL of clarificant into each tube.

b. Add 4 mL of chromogenic agent into each tube.

c. Fasten the tube mouth with fresh-keeping film, mix fully, and make a small hole in the film. Then incubate the tubes at 100℃ for 60 min.

d. Cool the tubes to room temperature with running water, centrifuge the tubes at 1600 g for 10 min.

e. Take 0.25 mL of the supernatant to the microplate with a micropipette (the precipitation cannot be added to the microplate).

f. Measure the OD value at 532 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Std1) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

Serum (plasma) sample:

$$\text{TBARS (μmol/L)} = (\Delta A - b) \div a \times f$$

Tissue sample:

$$\text{TBARS (μmol/gprot)} = (\Delta A - b) \div a \times f \div C_{pr}$$

[Note]

y: The absolute OD value of standard ($OD_{Standard} - OD_{Blank}$)

x: The concentration of standard;

a: The slope of standard curve;

b: The intercept of standard curve.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample (gprot/L)

ΔA: Absolute OD ($OD_{Sample} - OD_{Blank}$)

To easy calculate the test results, refer to the calculation file available on the webpage.

Example analysis

For mouse liver tissue, take 0.1 mL of 10% mouse tissue homogenate, carry the assay according to the operation steps. The results are as follows:

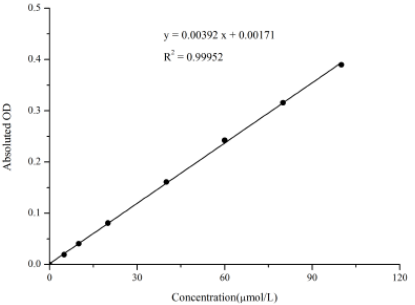
standard curve: y = 0.0038 x - 0.0013, the average OD value of the sample well is 0.106, the average OD value of the blank well is 0.047, the concentration of protein in sample is 15.18 gprot/L, and the calculation result is:

$$\text{TBARS content (μmol/gprot)} = (0.106 - 0.047 + 0.0013) \div 0.0038 \div 15.15 = 1.05 \text{ μmol/gprot}$$

Performance characteristics

- Standard curve (example)

The following data were obtained for the various standards over the range of 0–100 µmol/L standard.

Concentration (µmol/L)	OD at 532 nm	Standard Curve
100	0.434	
80	0.361	
60	0.287	
40	0.206	
20	0.126	
10	0.085	
5	0.064	
0	0.045	

- Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	5.9	25.70	76.50
%CV	4.5	4.2	4.2

CV = Coefficient of Variation

- Iner-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	5.9	25.70	76.50
%CV	6.8	7.2	7.3

CV = Coefficient of Variation

- Expected values

This assay was tested with rat serum, and plasma samples without dilutions.

Sample Type	Range (µmol/L)	Average (µmol/L)
Rat serum	15-21	18.4
Rat plasma	4-8	5.5

- Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 101%.

	Standard 1 (low conc.)	Standard 2 (middle conc.)	Standard 3 (high conc.)
Expected Conc. (μmol/L)	8.4	32.5	67.5
Observed Conc. (μmol/L)	8.5	32.0	69.7
Recovery rate (%)	101	98.5	103.2

- Recommended Plate Set Up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80
[Note]: A-H, standard wells; S1-S80, sample wells.												

- Sensitivity

The analytical sensitivity of the assay is 0.85 µmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

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