（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K298-F

Specification：48T(32 samples)/96T(80 samples)/ 500Assays (484 samples)

Measuring instrument：Fluorescence Microplate reader

（Ex/Em = 520 nm/550 nm）

Detection range：0.09-10 μmol/L

Elabscience® Thiobarbituric Acid Reactants (TBARS) Fluorometric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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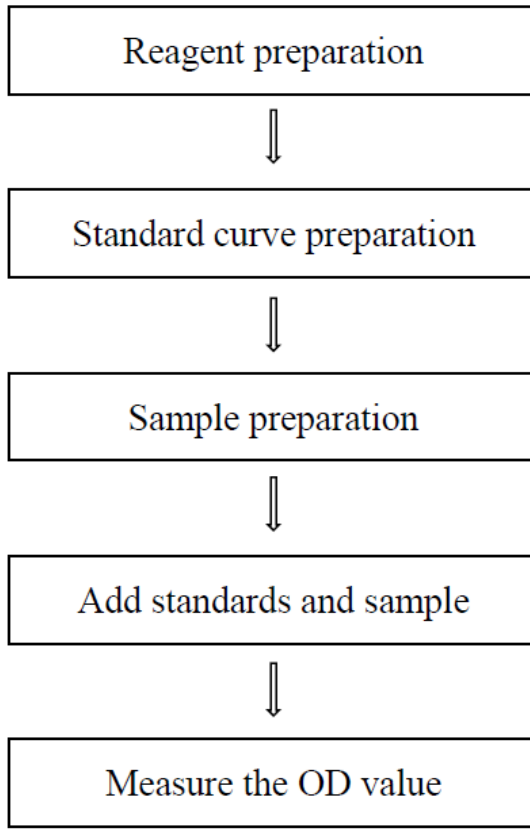
Assay summary

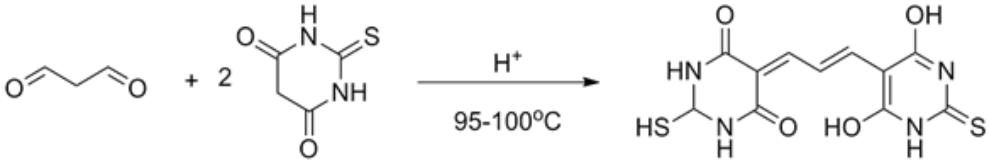


Intended use

This kit can be used to measure TBARS concentration in serum (plasma), animal tissue, culture cells and other samples.

Detection principle

TBARS and TBA can react under high temperature and acid conditions and then form a pink compound, the concentration of which is linearly related to the concentration of TBARS in the sample. The TBARS concentration can be calculated by measuring the fluorescence values at the excitation wavelength of 520 nm and the emission wavelength of 550 nm.



Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500Assays)	Storage
Reagent 1	Clarificant	12 mL × 1 vial	12 mL × 1 vial	60 mL × 1 vial	2-8 ℃, 12 months
Reagent 2	Acid Reagent	6 mL × 1 vial	12 mL × 1 vial	60 mL × 1 vial	2-8 ℃, 12 months
Reagent 3	TBA Reagent	Powder ×1 vial	Powder ×1 vial	Powder ×5 vials	2-8 ℃, 12 months shading light
Reagent 4	20 μmol/L Standard	5 mL × 1 vial	5 mL × 1 vial	12 mL × 2 vials	2-8 ℃, 12 months
	Black Microplate	96 wells			No requirement
	Plate Sealer	2 pieces			

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain

sufficient amount of reagents.

Materials prepared by users

Instruments:

Fluorescent Microplate reader(Ex/Em = 520 nm/550 nm), Vortex mixer, Magnetic Stirrers, Micropipettor, Thermostat water bath

Reagents:

Double distilled water, Acetic acid, normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Reagent preparation

① Frozen storage (2-8 ℃) may cause the clarificant to freeze. To re-dissolve place in a water bath (37 ℃) and until the clarificant looks clear. Equilibrate other reagents to room temperature before use.

② The preparation of acid application solution：
Dilute 1.2 mL acid reagent with 34 mL double-distilled water. The acid application solution should be prepared on spot. Store at 2~8 ℃ for 24 hours.

③ The preparation of TBA application solution：
Dissolve a vial of TBA reagent with 60 mL double distilled water (90-100 ℃) and mix fully. Then add 60 mL glacial acetic acid (self-prepared), mix fully and cool to room temperature. Store at 2~8 ℃ for 1 month protected from light.

④ The preparation of chromogenic agent：
For each tube, prepare 4 mL of chromogenic agent (mix well 3 mL of acid application solution, 1 mL of TBA application solution). The chromogenic agent should be prepared on spot and it must be use out in 24 hours.

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 20 μmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.5, 1, 2, 4, 6, 8, 10 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**0.5**	**1**	**2**	**4**	**6**	**8**	**10**
20 μmol/L standard (μL)	0	25	50	100	200	300	400	500
Double distilled water (μL)	1000	975	950	900	800	700	600	500

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cells:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 300 μL PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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

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

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① The temperature of water-bath and the time of incubation should be stabilized (95-100℃, 60 min).

② In the incubation of 100°C 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.

③ 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.

Operating steps

The measurement of samples

① Standard tube: Take 0.1 mL of standard solution with different concentrations into numbered 10 mL glass tubes.

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

② Add 0.1 mL of clarificant into each tube.

③ Add 4 mL of chromogenic agent into each tube.

④ Fasten the tube mouth with fresh-keeping film, mix fully, and make a small hole in the film. Then incubate the tubes at 100°C for 60 min.

⑤ Take the tubes out and put them in an ice bath to stop the reaction. After cooling to room temperature with running water, centrifuge the tubes at 1600× g for 10 min.

⑥ Take 0.25 mL the supernatant to the microplate with a micropipette (the precipitation cannot be added to the microplate).

⑦ Measure the fluorescence values at the excitation wavelength of 520 nm and the emission wavelength of 550 nm.

Note: Set control wells for whole blood, hemolysis serum and plasma samples, but not for normal serum and plasma.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean F value of the blank (Standard #①) from all standard readings. This is the absoluted F value.

3. Plot the standard curve by using absoluted F 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).

The sample:

1. Serum (plasma) sample:

$$\underset{(\mu mol/L)}{TABRS} = (\Delta F - b) \div a \times f$$

2. Tissue and cells sample:

$$\underset{(\mu mol/gprot)}{TABRS} = (\Delta F - b) \div a \times f \div C_{pr}$$

[Note]

f: Dilution factor of sample before test.

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

ΔF: Absolute fluorescence value of sample ($F_{Sample} - F_{Blank}$).

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	1.20	4.60	7.30
%CV	1.8	1.8	1.5

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	1.20	4.60	7.30
%CV	2.5	2.6	3.3

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 96%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	0.85	3.6	7.8
Observed Conc. (μmol/L)	0.8	3.5	7.6
recovery rate (%)	94.2	96	96.9

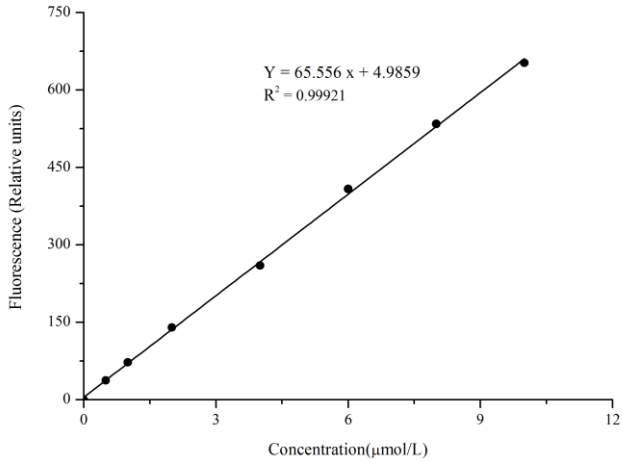
Sensitivity

The analytical sensitivity of the assay is 0.09 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.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	**0**	**0.5**	**1**	**2**	**4**	**6**	**8**	**10**
Average fluorescence value	50.2	87.5	122.7	190.1	310.0	458.5	584.4	702.8
Absoluted fluorescence value	0	37.3	72.5	140.0	260.0	408.3	534.3	652.6



Appendix II Example Analysis

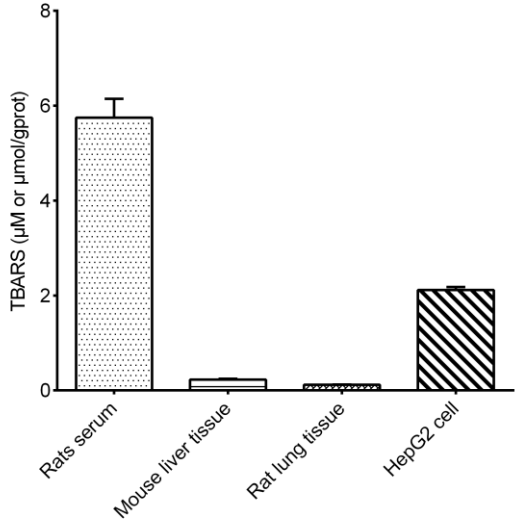
Example analysis：

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

standard curve: y = 63.104 x + 13.471, the average fluorescence value of the sample well is 70.527, the average fluorescence value of the blank well is 33.423, the concentration of protein in sample is 16.56 gprot/L, and the calculation result is:

$$\text{TBARS content (μmol/gprot)} = (70.527-33.423-13.471) \div 63.104 \times 10 \div 16.56 = 0.23\ \mu\text{mol/gprot}$$

Detect rat serum (dilute for 10 times), 10% rat liver tissue homogenate (the concentration of protein is 16.56 gprot/L, dilute for 10 times), 10% rat lung tissue homogenate (the concentration of protein is 5.04 gprot/L, dilute for 2 times) and HepG2 cell (the concentration of protein is 6.09 gprot/L) according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.