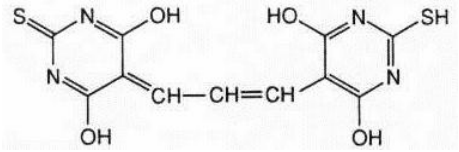
Universal TBARS Assay



A microplate assay for the determination of thiobarbituric acid reactive substances.

Flow Chart

Prepare Standards
↓
100 ul sample
↓
150 ul TBA solution
↓
150 ul Glacial Acetic Acid
↓
Incubate overnight at 75˚
↓
Centrifuge
↓
Remove 250 ul to black plate
↓
Read at Ex 484 nm Em 530 nm

***Alternately if using colorimetric assay transfer to clear plate and read at 530-540 nm in spectrophotometer**

**The Exocell TBARs kit is for Research Purposes only*

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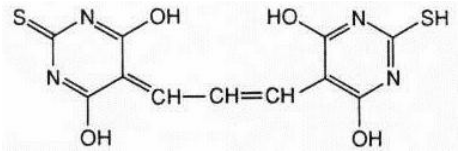
Cat. #1020
Version 2

The TBARS Assay kit measures malondialdehyde (MDA), a reactive compound formed from lipid peroxides that are generated under conditions of oxidative stress. Oxidative modification of lipids occurs with aging and various diseases, and increased oxidative stress is associated with diabetes and its complications. MDA forms an adduct with thiobarbituric acid (TBA). Results are calculated from a standard curve constructed with authentic MDA. Exocells TBARS assay can be used with a spectrum of biological samples including body fluids, tissue and cell specimens.

The sensitivity of measuring Thiobarbituric Acid Reactive Substances (TBARS) has made this assay the method of choice for screening and monitoring lipid peroxidation, a major indicator of oxidative stress. Oxidative stress in the cellular environment results in the formation of highly reactive and unstable lipid hydroperoxides. Decomposition of the unstable peroxides derived from polyunsaturated fatty acids results in the formation of malondialdehyde (MDA), which can be quantified colorimetrically following its controlled reaction with thiobarbituric acid. This assay remains the most widely employed assay used to determine lipid peroxidation.
We have modified this procedure for use with many types of samples including sera, plasma and human and animal biological tissues.

MDA-TBA Adduct



Assay Principle

The TBARS Assay kit is a tool for the direct quantitative measurement of MDA in biological samples. The samples are first reacted with TBA at 75˚C. After the overnight incubation the standards and samples are read either fluorometrically or spectrophotometrically. The MDA content in the samples is determined by linear regression comparison of the standard curve.

Reagents

Materials Supplied

Thiobarbituric Acid stock solution
TBARS Assay Diluent
MDA Standard (10 uM MalondialdehydeBis)
Acetic Acid
Microplate (1) for fluorometer

Materials required but not supplied.

Eppendorf tubes
Water bath or heat block set at 75˚
Adjustable pipettes
Graduated cylinders and assorted test tubes
Disposable gloves
96 well Fluorometer for reading samples/standards
Distilled H_2O
Spectrophotometer for reading colorimetrically
Protein Assay kit if doing ratio to protein

Storage

Store all kit reagents at 2-8˚C. The components should be used before the expiration date indicated on the outside of the box. TBA stock should be refrigerated.

Test Procedure

Step 1. Allow all reagents to reach room temperature. If using SDS for tissue lysis allow to sit 1 hour room temperature to dissolve SDS.

Step 2. Label Eppendorf tubes standard number and sample numbers

Step. 3. Resuspend the TBA reagent (100 mg in 1 ml DMSO) in 20 ml of diluent for use in assay.

Step. 4. Prepare samples 100 ul each in eppendorf tubes. Clarify sample either by centrifugation or filter. If SDS is being used for lysing, make sure sample is centrifuged at 14,000 x 10 minutes.

Step. 5. Prepare standard.
*Standard = 300 uM MDA

Number tubes 1 through 7. Add 100 ul MDA standard to tube 1. Add 100 ul of MDA diluent to tubes #2- #7. Add 100 ul MDA standard to tube #2 , mix , and remove 100 ul to tube #3, mix and remove 100 ul to tube #4, and so on.

Step. 6. Add 150 ul TBA solution to standard and sample tubes.

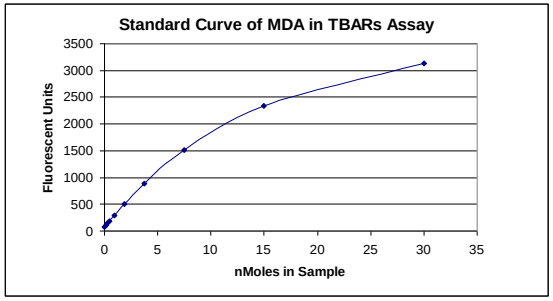
Step. 7. Add 150 ul of Acetic Acid solution to standard and sample tubes.

Step. 8. Vortex

Step. 9. Heat samples/standard overnight at 75ºC

Step. 10. Transfer 250 ul of standard and samples to 96 well black plate (supplied). Read samples in fluorometer at Ex 484 nm and Em 530 nm.

Alternately, if doing spectrophotometric measurement transfer 200 ul of standard and samples to 96 well clear plate(not supplied) and read absorbance at 532 nm.



Limitations:

1. Hemolyzed, icteric or grossly lipemic plasma samples are not suitable for use in TBARs analysis.
2. Non-lipid TBARs may be present in the sample. It is recommended that a sample with elevated TBARs levels be tested by HPLC.

References

1. Armstrong, D. and Browne, R. (1994). Free Radicals in Diagnostic Medicine. 366: 43-58.
2. Armstrong, D., et al. (1998). Free Radicals and Antioxidant Protocols. 108: 315-324.
3. Boyum, A. (1966). J. of Clinical Investigation. 21: Supplement 97.
4. Braun, D. and Fromherz, P. (1997). Applied Physics A.
5. Gidez, L., et al. (1982). J. of Lipid Research. 23: 1206-1223.
6. Lef'evre G., et al. (1998). Annals de Biologie Clinique. 56(3): 305-319.
7. Ohkawa, H., et al. (1979). Anal. Biochem. 95: 351-358. 8. Yagi, K. (1998). Free Radicals and Antioxidant Protocols. 108: 101-106.