Malondialdehyde (MDA)

Assay kit

(96/48 Tests)

ZellBio GmbH (Germany)

CAT No. ZB-MDA-96A

CAT No. ZB-MDA-48A

Sample Types Validated

Serum, Plasma, Saliva, Cell Culture Supernatant, Tissue Homogenate and Other Related Biological Liquid

Please read this insert completely prior to using the product.

For Research Use Only, Not For in vitro Diagnostic use

Related Products

	ZellBio Antioxidant	Cat No.
1	Total Antioxidant Capacity (TAC)	ZB-TAC-48A/ ZB-TAC-96A
2	Glutathione Reductase (GR)	ZB-GR-48A/ ZB-GR-96A
3	Malondialdehyde (MDA)	ZB-MDA-48A/ ZB-MDA-96A
4	Catalase (CAT)	ZB-CAT-48A/ ZB-CAT-96A
5	Superoxide Dismutase (SOD)	ZB-SOD-48A/ ZB-SOD-96A
6	Glutathione (GSH)	ZB-GSH-48A/ ZB-GSH-96A
7	Glutathione peroxidase (GPX)	ZB-GPX-48A/ ZB-GPX-96A
8	Nitric Oxide (NO)	ZB-NO-48A/ ZB-NO-96A
9	Hydrogen Peroxide (H_2O_2)	ZB-HPO-48A/ ZB-HPO-96A
10	Vitamin C (Vit C)	ZB-VITC-48A/ ZB-VITC-96A
11	Vitamin E (Vit E)	ZB-VITE-48A/ ZB-VITE-96A
12	Paraoxonase (POX)	ZB-POX-48A/ ZB-POX-96A
13	Xanthine Oxidase (XOX)	ZB-XOX-48A/ ZB-XOX-96A
14	Total Oxidant Status (TOS)	ZB-TOS-48A/ ZB-TOS-96A
15	Total Polyphenol Content (TPC)	ZB-TPC-48A/ ZB-TPC-96A

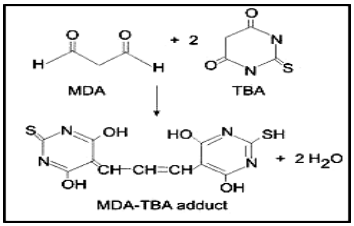
ZellBio GmbH assay kit is used to **quantitative** assay Human/Animal/Biological samples MDA on the basis of colorimetric (**535 nm**) method. **ZellBio GmbH** microplate format kit is for research only and is not for use in diagnostic procedures.

Intended Use

ZellBio GmbH Malondialdehyde (**MDA**)/ Thiobarbituric Acid Reactive Substances (**TBARS**) assay kit is a simple, reproducible, and standardized tool for assessment of lipid peroxidation in biological samples, e.g. **plasma, serum, saliva, urine, tissue homogenates, cell lysates, and other related biological liquid.**

Test principle

ZellBio GmbH assay kit uses the MDA-TBA adduct formed by the reaction of Malondialdehyde (MDA) and thiobarbituric acid (TBA) under high temperature. Malondialdehyde is measured in acidic media and heat (90-100°C) colorimetrically at **535** (**530-540 nm**).



Materials supplied in the Test Kit

	Kit Contents	96 Tests	48 Tests
1	Reagent 1	Powder	Powder
2	Reagent 2 (5X)	11 mL	6 mL
3	Reagent 3 (10X) (Alkali)	5.5 mL	3 mL
4	Reagent 4	6 mL	3 mL
5	Standard 5000 µM **(Store at -20ºC)**	70 µL	70 µL
6	Microplate	1	1
7	User Manual	1	1

Materials Required but not supplied

1. Boiling water bath
2. Microplate/ELISA reader
3. Precision pipettes and Disposable pipette tips
4. Double distilled water
5. Disposable tubes for sample preparation

Important Notes

1. Before opening the kit kept at the temperature of 2-8˚C (the standard at -20˚C), it takes at least 30 minutes to increase naturally to room

temperature.

2. When adding samples, sample injector must be used for each time and should also be frequently checked for its precision to avoid individual error.
3. The instruction must be strictly followed while the reading of Microplate/ELISA reader must be set as the standard of determining the experiment result.
4. Pipette tips in hand should not be used more than once in order to avoid cross contamination.
5. Reagents of different batches must not be mixed and should be used before their respective validity dates.

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Human MDA were tested 20 times on a microplate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Human MDA were tested on 3 different plates, 8 replicates in each plate.

$$CV\ (\%) = (SD/mean) \times 100$$

Intra-Assay: CV 5.8%

Inter-Assay: CV 7.6%

Specimen Requirements

1. After collecting the sample, extraction should be immediately carried out in accordance with related documents. After extraction, experiment should be conducted immediately as well. Otherwise, keep the sample at -20˚C. Or deeper at -70 or -80˚C. Avoid repeated freeze-thaw cycles.
2. **Serum**: Allow the serum to clot for 5-10 minutes at room temperature. Centrifuge (at 2000-4000 RPM) for 10 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.
3. **Plasma**: In accordance with the requirements of sample collection, EDTA or sodium citrate should be used as anti-coagulation. Add EDTA or sodium citrate and mix them for a minute. Centrifuge (at 2000-4000 RPM) for approximately 10 min. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.
4. **Cell culture supernatant**: Collect by sterile tubes when examining secrete components. Centrifuge (at 2000-3000 RPM) for approximately 10 minutes. Collect the supernatants carefully. When examining the components within the cell, use PBS (pH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells through repeated freeze-thaw cycles to let out the inside components. Centrifuge (at 2000-3000 RPM) for approximately 10 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be

performed again.

5. **Tissue sample**: Incise sample and weigh up. Add a certain amount of PBS (100mM, pH 7.4) for homogenization or freeze with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8˚C and add a certain amount of PBS (pH 7.4) and then homogenize the sample (~100 mg tissue per 1 mL PBS buffer) thoroughly by hand or homogenizer. Centrifuge (at 4000-6000 RPM) for approximately 20 minutes. Collect the supernatants carefully. Aliquot and keep one for examination and freeze the others for later use. Alternative method for tissue preparation is: Prior to dissection, perfuse tissue with a PBS (phosphate buffered saline) solution, pH 7.4, containing 0.16 mg/ml heparin to remove any red blood cells and clots. Homogenize the tissue in 5-10 ml of cold buffer (i.e., 50 mM Tris-HCl, pH 7.5, 5 mM EDTA, and 1 mM DTT) per gram tissue. Centrifuge at 10,000 x g for 15 minutes at 4°C. Remove the supernatant for assay and store on ice. If not assaying on the same day, freeze the sample at -80°C. The sample will be stable for at least one month.

Reagent Preparation

1) Ready R2: For 96 tests, add 44mL distilled water to 11mL R2. For 48 tests, add 24mL distilled water to 6mL R2.
2) Ready R3: For 96 tests, add 49.5mL distilled water to 5.5mL R3. For 48 tests add 27mL distilled water to 3mL R3.
3) Chromogenic reagent: Mix powder of R1 with 55mL R2 ready

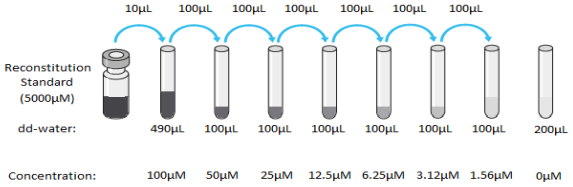
reagent and 55mL R3 ready reagent for 96 tests and Mix powder of R1 with 30mL R2 ready reagent and 30mL R3 ready reagent for 48 tests. Warm slowly until powder dissolves completely. This reagent stable for a day. So, it can prepare the Chromogenic solution as it needs with the same proportion.

Standards preparation

Standard solutions: The stock standard is 5000μM; At first by 1:50 dilution, prepare 100μM with dd-water, then by serial dilution prepare 100μM, 50μM, 25μM, 12.5μM, 6.25μM, 3.12μM, 1.56μM and 0μM (dd-water).

Dilution of standard solutions: (This kit has a standard of original concentration, which could be diluted in small tubes by user independently following the instruction.):

100μM	Standard working	10μL Original Standard + 490μL dd-water
50μM	Standard No.6	100μL Standard working + 100μL dd-water
25μM	Standard No.5	100μL Standard No.6 + 100μL dd-water
12.5μM	Standard No.4	100μL Standard No.5 + 100μL dd-water
6.25μM	Standard No.3	100μL Standard No.4 + 100μL dd-water
3.12μM	Standard No.2	100μL Standard No.3 + 100μL dd-water
1.56μM	Standard No.1	100μL Standard No.2 + 100μL dd-water
0μM	Standard No.0	200μL dd-water

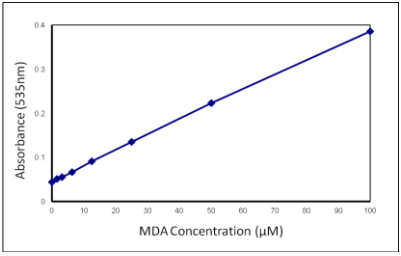


Assay procedure

1. All reagents (except samples) must be equilibrated to room temperature (RT). Shake the unknown sample for homogenation well.
2. Add 50μL standards/samples to related name test tubes.
3. Add 50μL R4 reagent (if it is cloudy, warm until to become a clear solution).
4. Add 1mL ready Chromogenic solution.
5. Heat the mixture for one hour in boiling water bath (pink color formation).
6. Cool the above tests tube in ice bath and centrifuge them 10min around 3000-4000 rpm.
7. Pipette 200μL of pink color supernatant into the microplate.
8. Read the absorbance with microplate reader/ELISA reader at 535nm.
9. Calculate MDA level in unknown samples based on standard curve which drown using standard points' absorbance.

Calculation

Make concentration of standards the abscissa and OD value the ordinate. Draw the standard curve on the coordinate paper. According to the OD value of the sample, locate its corresponding concentration which is the concentration of the sample; or calculate the linear regression equation of standard curve according to the concentration of the standard and the OD value. Then substitute with the OD value of the sample to calculate its concentration.



A typical standard curve of ZellBio GmbH **MDA** Assay kit

Assay range

ZellBio GmbH MDA assay kit can be used for MDA determination in the range of **0.78 - 50µM.**

Sensitivity

ZellBio GmbH MDA assay kit can determine MDA in biological samples with **0.1µM** sensitivity.

Validity & Storage

Two years at 2-8˚C (Standard at -20˚C) for intact kit, See label on the outer box for the specific date.

Summary

Prepare reagents, samples and standards.

Add 50µL standards/samples to related name test tubes

Add 50µL R4 reagent and mix

Add 1mL Ready Chromogenic solution and Heat the mixture for one hour in boiling water bath

Cool the tests tube in ice bath and centrifuge them 10min around 3000-4000 rpm.

Pipette 200µL of pink color supernatant into the microplate and Read the absorbance at 535nm

Calculate

References:

1. Dawn-Linsley, M., Ekinci, F.J., Ortiz, D., et al. Monitoring thiobarbituric acid- reactive substances (TBARs) as an assay for oxidative damage in neuronal cultures and central nervous system. J. Neurosci. Meth. 141, 219-222 (2005).
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4. Armstrong, D. and Browne, R. The analysis of free radicals, lipid peroxides, Glutathione antioxidant enzymes and compounds to oxidative stress as applied to the clinical chemistry laboratory. Free Radicals in Diagnostic Medicine 366, 43-58 (1994).
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6. Dawn-Linsley, M., Ekinci, F.J., Ortiz, D., et al. Monitoring thiobarbituric acid-reactive substances (TBARs) as an assay for oxidative damage in neuronal cultures and central nervous system. J. Neurosci. Meth. 141, 219-222 (2005).
7. Satoh, K. (1978). Serum lipid peroxide in cerebrovascular disorder determined by a new colorimetric method. Clin. Chim. Acts 90:37-43.

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