KAMIYA BIOMEDICAL COMPANY

Metallothionein (MT) I / II ELISA

For the simultaneous determination of metallothioneins I and II in serum, plasma, urine, cell homogenates and cell culture supernatant

Cat. No. KT-1874

For research use only. Not for use in diagnostic procedures.

PRODUCT INFORMATION

Metallothionein (MT) I / II ELISA
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INTENDED USE
The Metallotionein (MT) I / II ELISA is for the simultaneous determination of metallothioneins I and II in serum, plasma, urine, cell homogenates and cell culture supernatant. For research use only. Not for use in diagnostic procedures.

INTRODUCTION
Metallothionein (MT), which is Cd binding protein, was discovered from horse kidney by Margoshe and Vallee in 1957. MT is present in all animal cells and has strong affinity against another heavy metal. The physiological role of MT is related to detoxication of organic metal, metabolic regulation of bioessential metal and protection from stress and free radicals.

Obtaining high affinity anti-MT antibody was very hard because MT is a cysteine rich (about 30%), low molecular weight protein and lacks aromatic amino acid in its structure. Therefore, the high sensitive ELISA system was not established and it was difficult to investigate the behavior of MT *in vivo*.

We developed an anti-MT antibody and established a high sensitive ELISA system for determining human and animal MT. The epitope of this antibody is located at the NH_2 terminal acetylated peptides in MT, enabling the assay of native MT. In addition, the NH_2 terminal of MT is a highly conserved sequence in many animal species, therefore, the amount of MT in almost all animals can be measured in this ELISA kit.

PRINCIPLE
This ELISA kit adopts the indirect competitive reaction using a rabbit MT polyclonal antibody which recognizes the N-terminal of MT. The MT is coated on the surface of the 96 well plate, onto which MT calibrator or samples to be measured and anti-MT antibody are overlaid and incubated. After a washing step, HRP-conjugated anti-rabbit antibody is added and incubated again. HRP conjugated antibody and anti-MT antibody – MT complexes are formed. After washing, substrate is added, which reacts with the HRP conjugate to produce blue color. More blue color means less MT. The optical densities are measured and plotted to calculate the exact concentration of MT.

The antibody in this kit can recognize the N terminal, especially the acetyl group of N-terminal amino acid. Therefore, the cross reactivity against recombinant MT protein expressed in bacteria is under 5%.

The kit is for the quantitative detection and the procedure of this assay is simple, easy and hardly influenced by physiological active substances and body fluid.

The epitope of the antibody we used in this kit is located at NH_2 terminal and acetylated peptides in MT. MT I and II which have similar NH_2 terminal sequences can react against this antibody.

COMPONENTS
1) Antigen coated 96 well plate, 1 plate, MT coated plate
2) MT calibrator, 1 mL x 1 at 10 µg/mL, Rabbit MT
3) Calibrator diluent, 25 mL x 1, Phosphate buffered saline (PBS)
4) Antibody solution, 6 mL x 1, Phosphate buffered saline containing Anti-MT antibody
5) Secondary antibody, 120 µL x 1, HRP-conjugated antibody
6) Secondary antibody diluent, 12 mL x 1, PBS
7) Enzyme Substrate, 12 mL x 1, TMB
8) Stop solution, 6 mL x 1, 1 N H_2SO_4
9) Concentrated washing solution, 50 mL x 1, 0.2% Tween 20 with 10X phosphate buffered saline
10) Plate seal

MATERIALS REQUIRED BUT NOT PROVIDED

1) Refrigerator (4℃) for incubation.
2) Spectrophotometer (microtiter plate reader with 450 nm filter) equipped with micro-titer plate
3) Microtiter plate shaker
4) Microtiter plate washer (or wash bottle)
5) Micro-pipette (50 and 100 µL) and tips
6) Graduated cylinder (1,000 mL)
7) Distilled water or de-ionized water

PRECAUTIONS

1) Reagents should be diluted immediately before use, and clean containers should be used in every step of preparation.
2) Refrigerate (4℃) the kit after opening and use within 1 week.
3) Precipitate may appear in the concentrated washing solution during storage, and can be dissolved during the dilution process.
4) Pipetting into wells should accurately be carried out as the pipetting is responsible for the overall accuracy of estimation. New tips should be used for each sample to avoid possible contamination.
5) Highly concentrated (>1,000 ng/mL) samples should be diluted with calibrator diluent.
6) Every sample should be run in duplicate.
7) After stopping the reaction, carry out the OD determination immediately.
8) The calibration curve should be made for each assay because the level of photometric reaction can be slightly affected by reaction temperature, time course and degree of mixing.
9) Careful avoidance of strong light during estimation and storage is recommended.
10) Do not combine kits from different lots.
11) This kit is for research use only. Not for use in diagnostic procedures.

REAGENT PREPARATION

1) Dilute the calibrator solution (10,000 ng/mL) with calibrator diluent by 1/5 and prepare as follows; 10,000, 2,000, 400, 80, 16, 3.2, 0.64 ng/mL. Calibrator diluent is used as the 0 ng/mL calibrator.
2) Anti-MT antibody solution is already prepared. Ready to use.
3) The HRP conjugated secondary antibody (120 µL) should be diluted with secondary antibody diluent and be used after thoroughly mixing.
4) The enzyme substrate should be used after equilibration at room temperature.
5) Stop solution is already prepared. Ready to use.
6) The concentrated washing solution (50 mL) should be diluted with distilled water (450 mL), and be used to wash the plates.

STORAGE

Store at 4℃.

SPECIMEN COLLECTION AND HANDLING

Animal samples, tissue or cell are homogenized with PBS and centrifuged at 10,000 rpm for 30 min. The supernatant is collected and diluted with PBS according to need.
In the case of serum or plasma assay, use Microcon Ym-100 (amicon) to filter the proteins, M.W. under 100 kDa. Centrifuge at 10,000 rpm for 30 min. and collect the filtrated (flow-through) serum or plasma, without the protein M.W. above 100 kDa. This flow-through plasma or serum is ready to use for assay.

ASSAY PROTOCOL

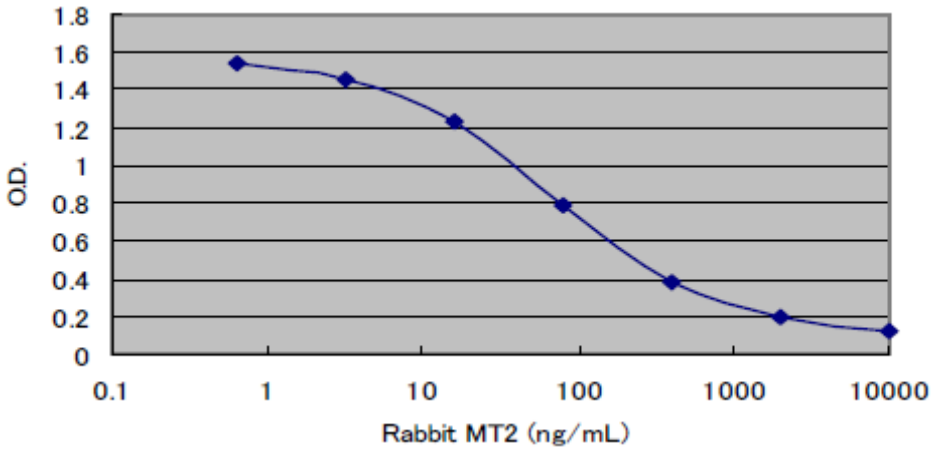
1) **Keep the buffer (diluted calibrators, samples and antibody solution) and the plate condition under 4℃ while working. Recommend the plate, samples and buffer be kept on ice. Do not equilibrate to room temp!**
2) Take out required number of the strips from the plate. Wash the strips 1 time with washing buffer, then turn the wells upside-down and tap out on a paper towel until the remaining buffer has been removed.
3) Add 50 µL of calibrator or assay sample into each well and then add the antibody solution (50 µL) to the same wells.
4) The contents in the wells are stirred by tapping the plate with fingers (a plate shaker can also be used).
5) Cover the plate and incubate at 4℃ (in refrigerator) for 1 hour to maintain a steady progress of reaction.

6) After incubation, discard the solution from the wells and fill the washing solution. Wash 3 times with the washing buffer solution, then turn the wells upside-down and tap out on a paper towel until the remaining buffer has been removed. When an automatic washer is used, set 3 times wash with the washing solution (350 µL).
7) Add secondary antibody solution (120 µL) to the secondary antibody diluent, and mix by swirling the bottle prior to use.
8) Add 100 µL of the diluted secondary antibody solution (prepared in step 7) to each well.
9) Repeat step 4.
10) Seal the strips and incubate for 1 hour at room temp.
11) Wash the strips in the same way as step 6.
12) Add 100 µL of the substrate in each well and incubate for 10 min. at room temp.
13) Add 50 µL of the stop solution into each well.
14) Measure the optical density (OD) of the wells containing the 8 calibrator concentrations of MT using a spectrophotometer (microtiter plate reader with 450 nm filter).

RESULTS

Calibration curve is obtained by plotting the OD values versus the calibrator concentrations of MT. Concentration of MT in the samples can be read from the calibration curve (recommend 4-parameter logistic model).

Example calibration curve. Do not use to calculate results.



FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com