Human Metallothionein (MT) ELISA Kit

to determine Human Metallothionein in Serum, Blood Plasma, Saliva, Urine,

And Other Related Tissue Liquid Samples.

INSTRUCTION MANUAL

FOR ELISA KIT No: LTuH3371EA

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INTENDED USE

This kit is used to assay the Human Metallothionein (MT) in the sample of serum, blood plasma, saliva, urine, and other related biological liquid. For *in vitro* use only.

MANUAL VERSION 1.02

BACKGROUND

Metallothionein (MT) is a family of cysteine-rich, low molecular weight (MW ranging from 500 to 14000 Da) proteins. They are localized to the membrane of the Golgi apparatus. MTs have the capacity to bind both physiological (such as zinc, copper, selenium) and xenobiotic (such as cadmium, mercury, silver, arsenic) heavy metals through the thiol group of its cysteine residues, which represent nearly 30% of its constituent amino acid residues.

PRINCIPLE

Human Metallothionein (MT) ELISA Kit employs a two-site sandwich ELISA to quantitate Metallothionein in samples. An antibody specific for Metallothionein has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Metallothionein present is bound by the immobilized antibody. After removing any unbound substances, HRP-Conjugate Human Metallothionein detection antibody is added to the wells. Following a wash to remove any unbound HRP reagent, a Chromogen solution is added to the wells and color develops in proportion to the amount of Metallothionein bound in the initial step. The color development is stopped and the intensity of the color is measured.

MATERIALS SUPPLIED IN THIS KIT

Human Metallothionein microplate: 96 well polystyrene microplates (8 strips of 12 wells) coated with the antibody specific for Human Metallothionein.

Human Metallothionein standard: Human Metallothionein in a buffered protein base with preservatives, liquid.

Standard diluent: Diluent solution for reconstituted standard.

Sample diluent: Diluent solution for reconstituted samples.

HRP-Conjugate Human Metallothionein detection antibody: Antibody specific for Human Metallothionein, liquid.

Chromogen solution A: liquid.

Chromogen solution B: liquid.

Stop solution: liquid.

Wash buffer: 30X liquid.

Plate covers.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator 2. Microplate reader 3. Precision pipettes and tips
4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

IMPORTANT NOTES

1. Before using, keep the kit outside and allow it to come to room temperature.
2. After breaking the seal of ELISA coated-plate, keep the unused strips in the zipper bag at 2-8 °C.
3. Pipette tips and seal plate membrane should not be used more than once in order to avoid cross contamination.
4. All samples and all discard generated should be disposed as per local rules.
5. Reagents of different batches must not be mixed and should be used before their respective validity dates.
6. Substrate B is sensitive to light and therefore should not be exposed to light for too long.

PRECISION

Intra-assay Precision (Precision within an assay): 4 samples with low, middle and high level high level Human MT were tested 20 times on one plate, respectively.
Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level high level Human MT were tested on 3 different plates, 8 replicates in each plate.
CV(%) = SD/meanX100
Intra-Assay: CV<9%
Inter-Assay: CV<11%

Recovery: The recovery of Human Metallothionein spiked to different levels in samples throughout the range of the assay in various matrices was evaluated.
The recovery ranged from 98% to 116% with an overall mean recovery of 106%

Sensitivity: The minimum detectable dose (MDD) of Human Metallothionein is typically less than 25 ng/L. The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity: Human Metallothionein ELISA Kit can be used to measure Human Metallothionein in samples. Human Metallothionein ELISA Kit has high sensitivity and excellent specificity for detection of Human Metallothionein. No significant cross-reactivity or interference between Human Metallothionein and analogues was observed.

SAMPLE PREPARATION

1. Samples containing NaN_3 are not recommended for testing with ELISA as they may inhibit the activity of Horse Radish Peroxidase (HRP).

2. After extraction, experiment should be conducted immediately. Otherwise, keep the sample at -20 °C. Avoid repeated freeze-thaw cycles.

3. Serum: Allow the sample to clot for 10-20 minutes at room temperature. Centrifuge (at 2000-3000 RCF) for 20 minutes. Collect the supernatant carefully. In case of storage, centrifugation should be performed again prior to use.

4. Blood plasma: During sample collection EDTA or sodium citrate should be used for anti-coagulation. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.

5. Urine: Collect the sample in a sterile tube. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use. When collecting pleuroperitoneal fluid and cerebrospinal fluid, please follow a similar procedure.

6. Cell culture supernatant: For secreted components, centrifuge (at 2000-3000 RCF) for approximately 20 minutes and 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 by repeated freeze-thaw cycles to let out the inside components. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes and collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.

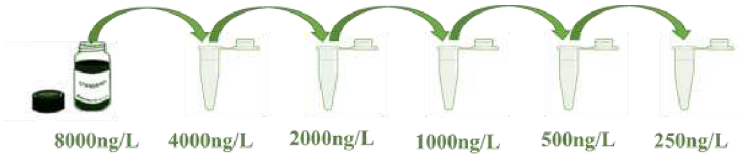
7. Tissue sample: Incise tissue sample of interest and add few mls of PBS (pH 7.4). Freeze with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8 °C. Add few mls of PBS (pH 7.4) and then homogenize the sample thoroughly by hand or by homogenizer. Centrifuge (at 2000-3000 RCF) for

approximately 20 minutes. Collect the supernatants carefully. Aliquot and keep one for examination and freeze the others for later use.

ASSAY PROCEDURE

1. Dilution of standard solutions: This kit contains a standard of known concentration, which could be diluted in small tubes by the end-user by following the instruction in the table below:

4000ng/L	Standard No.5	150µl Original Standard + 150µl Standard diluents
2000ng/L	Standard No.4	150µl Standard No.5 + 150µl Standard diluents
1000ng/L	Standard No.3	150µl Standard No.4 + 150µl Standard diluent
500ng/L	Standard No.2	150µl Standard No.3 + 150µl Standard diluent
250ng/L	Standard No.1	150µl Standard No.2 + 150µl Standard diluent



2. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the microplate.

3. Add standard: Set Standard wells, testing sample wells. Add diluted standard 50µl to standard well.

4. Add Sample: Add sample diluent 40 µl to testing sample well. Then add sample 10µl to testing sample well; for Blank well don't add anything.

5. Cover with a plate cover and incubate for 45 minutes at 37 °C.

6. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash buffer (250 µl) using a squirt bottle,

manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

7. Add 50 µl HRP-Conjugate to each well, except blank well.

8. Cover with a new adhesive strip. Incubate for 30 minutes at 37 °C.

9. Repeat the aspiration/wash process for five times as in step 5.

10. Add 50 µl chromogen solution A and 50 µl chromogen solution B to each well. Gently mix and incubate for 15 minutes at 37°C. Protect from light.

11. Add 50µl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

12. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

PROTOCOL SUMMARY

Prepare reagents, samples and standards.

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Add prepared samples and standards in respective wells together and incubate for 45 minutes at 37 °C.

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Wash the plate five times. Add HRP-Conjugate and incubate for 30 minutes at 37 °C.

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Wash the plate five times. Add Chromogen solution A and B. Incubate for 15 minutes at 37 °C for color development and add stop solution.

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Read the OD value within 15 minutes.

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Plot and Calculate.

CALCULATION

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration for each standard on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Human Metallothionein concentrations versus the log of the O.D. and the best

fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be
multiplied by the dilution factor.

ASSAY RANGE： 250ng/L－4000ng/L.

PACKAGE SIZE： 96Tests.

SENSITIVITY： <25ng/L.

LINEARITY: To assess linearity of the assay, samples containing and/or spiked with high concentrations of Human Metallothionein were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

VALIDITY & STORAGE: Twelve months (at 2-8°C, unopened).