Version 22-04/24

User's Manual

Human MCM3 ELISA Kit

REF **DEIA-LL100**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

For quantitative detection of MCM3 in serum, plasma, tissue homogenatesand other biological fluids.

Principles of Testing

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti-MCM3 antibody was pre-coated onto 96-well plates. And the biotin conjugated anti- MCM3 antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRPStreptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the MCM3 amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of MCM3 can be calculated.

Reagents And Materials Provided

No.	Item	Specifications(96T)	Storage
E001	ELISA Microplate(Dismountable)	8×12	2-8°C/-20°C
E002	Lyophilized Standard	2vial	2-8°C/-20°C
E039	Sample/Standard Dilution Buffer	20ml	2-8°C
E003	Biotin-labeled Antibody(Concentrated)	120ul	2-8°C(Avoid Direct Light)
E040	Antibody Dilution Buffer	10ml	2-8°C
E034	HRP-Streptavidin Conjugate(SABC)	120ul	2-8°C(Avoid Direct Light)
E049	SABC Dilution Buffer	10ml	2-8°C
E024	TMB Substrate	10ml	2-8°C(Avoid Direct Light)
E026	Stop Solution	10ml	2-8°C
E038	Wash Buffer(25X)	30ml	2-8°C
E006	Plate Sealer	5pieces	
E007	Product Description	1copy	

Materials Required But Not Supplied

1. Microplate reader (wavelength:450nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Storage

2-8 °C for 6 months

Specimen Collection And Preparation

Isolate test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

1. Serum:

Place whole blood sample at room temperature for 2 hours or put it at 4°C overnight and centrifugation for 20 minutes at approximately 1000×g, collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

2.Plasma:

Collect plasma using EDTA-Na_2 as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

3.Tissue Homogenates:

As hemolysis blood has relation to assay result, it is necessary to remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Mince tissue after weighing it and get it homogenized in PBS (the volume depends on the weight of the tissue. Generally speaking, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5minutes at 5000×g to get the supernate.

4.Cell Culture supernate:

Centrifuge supernatant for 20 minutes at 1000×g at 2 - 8°Cto remove insoluble impurity and cell debris. Collect the clear supernate and carry out the assay immediately.

5.Other Biological Fluids:

Centrifuge samples for 20 minutes at 1000×g at 2-8°C. Collect supernatant and carry out the assay immediately.

6.Sample Preparation:

Samples shall be clear and transparent and remove suspended solids by centrifugation.

Note: Samples to be used within 5 days can be stored at 4°C, besides that, samples must be stored at -20°C (assay ≤1 month) or -80°C(assay≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for this assay.

Sample Dilution Guideline

End usershould estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be making in pre-experiment.The following dilution solutions are for reference only:

1. High concentration (100-1000ng/ml): Dilution: 1:100. (i.e. Add 1µl of sample into 99µl of Sample/Standard

Dilution Buffer.)

2. Medium concentration (10-100ng/ml): Dilution: 1:10.(i.e. Add 10µl of sample into 90µl of Sample/Standard Dilution Buffer.)

3. Low concentration (0.156-10ng/ml): Dilution: 1:2. (i.e. Add 50µl of sample into 50µl of Sample/Standard Dilution Buffer.)

4. Very low concentration (≤0.156ng/ml): Unnecessary to dilute, or dilute at 1:2.

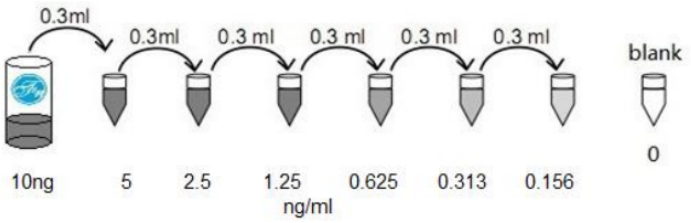
Reagent Preparation

Put the kit at room temperature for 20 minutes before use.

1. Wash Buffer: Dilute 30mL Concentrated Wash Buffer into 750 mL Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.
2. Standard:

1) 10ng/ml of standard solution: Add 1 ml Sample / Standard dilution buffer into one Standard tube, keep the tube at room temperature for 10 minutes and mix them thoroughly.

2) 5ng/ml→0.156ng/ml of standard solutions: Label 6 Eppendorf tubes with 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml, 0.312ng/ml, 0.156ng/ml, respectively. Add 0.3 ml of the Sample/Standard dilution buffer into each tube. Add 0.3 ml of the above 10ng/ml standard solution into 1st tube and mix them thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix them thoroughly, and so on.



Note: It is best to use Standard Solutions within 2 hours. The Standard Solution shall be at 4°C up to12 hours. Or store it at -20 °C up to 48 hours. Avoid repeated freeze-thaw cycles.

3. Preparation of Biotin-labeled Antibody Working Solution Prepare it within 1 hour before experiment.

1) Calculate required total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

2) Dilute the Biotin-detectionantibody with Antibody Dilution Bufferat 1:100 and mix them thoroughly. (i.e. Add 1µl Biotin-labeled antibody into 99µl Antibody Dilution Buffer.)

4. Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution: Prepare it within 30minutes before experiment.

1) Calculate required total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

2) Dilute the SABC with SABC Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1µl of SABC into 99 µl of SABC Dilution Buffer.)

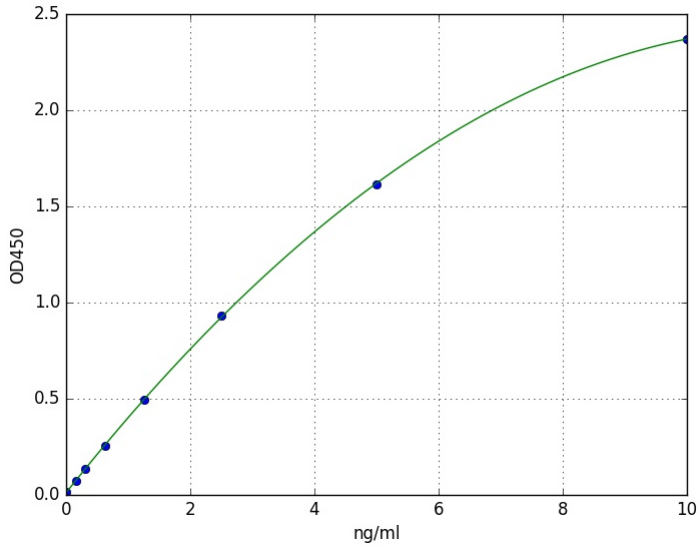
Assay Procedure

Before adding reagents into wells, equilibrate TMB Substrate for 30 min at 37 °C. When diluting samples and reagents, they must be mixed completely and evenly. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommended to measure each standard and sample in duplicate. Wash plate 2 times before adding standard, sample and control (zero) wells!
2. Aliquot 0.1ml of10ng/ml,5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml, 0.312ng/ml, 0.156ng/ml, standard solutions into the standard wells.
3. Add 0.1 ml of Sample/Standard Dilution Buffer into the control (zero) well.
4. Add 0.1 ml of properly diluted sample (Humanserum, plasma, tissue homogenatesand other biological fluids) into test sample wells.
5. Seal the plate with a cover and incubate at 37 °C for 90 minutes.
6. Remove the cover and discard the plate content, and wash plate 2 times with Wash Buffer.Do NOT let the wells dry completely at any time.
7. Add 0.1 ml Biotin-labeled antibody working solution into above wells (standard, test sample & zero wells). Add the solution at the bottom of each well without touching the sidewall.
8. Seal the plate with a cover and incubate at 37°C for 60 min.
9. Remove the cover, and wash plate 3 times with Wash Buffer,and let the wash buffer stay in the wells for 1 minute each time.
10. Add 0.1 ml of SABC Working Solution into each well, cover the plate and incubate at 37°C for 30 minutes.
11. Remove the cover and wash plate 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minute each time.
12. Add 90µl TMB Substrate into each well, cover the plate and incubate at 37°Cin dark within 15-30 minutes. (**Note**: This incubation time is for reference only, end user shall determine the optimal time.) It will turn blue in the first 3-4 wells (with most concentrated MCM3 standard solutions), the other wells may not display obvious color.
13. Add 50µl Stop Solution into each well and mix them thoroughly. The color changes to yellow immediately.
14. Read the O.D. absorbance at 450 nm in Microplate Reader immediately after adding the stop solution. Regarding calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). TheMCM3concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Typical Standard Curve



Precision

Intra-assay:CV<8%;

Inter-assay:CV<10%

Detection Range

0.156-10ng/ml

Sensitivity

< 0.094ng/ml

Specificity

This assay has high sensitivity and excellent specificity for detection ofMCM3. No significant cross-reactivity or interference betweenMCM3and analogues was observed. **Note**: Limited by current skills and knowledge, it is difficult for us to complete the crossreactivity detection betweenMCM3and all the analogues, therefore, cross reaction may still exist.

Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.

5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Avoid using the reagents from different batches together

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