Gentaur Molecular Products BVBA
Address: Voortstraat 49, 1910 Kampenhout, Belgium
T: 0032 16 58 90 45 | **E:** info@gentaur.com
Websites: www.gentaur.com | www.maxanim.com

Catalog No: YLA1817MO

96 Test

Mouse symmetric dimethylarginine(SDMA)ELISA Kit

FOR RESEARCH USE ONLY; NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!
PLEASE READ THROUGH ENTIRE procedure BEFORE BEGINNING!

INTENDED USE

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of SDMA in Mouse serum 、plasma、tissue homogenates and other biological fluids.

REAGENTS AND MATERIALS PROVIDED

Reagents	Quantity	Reagents	Quantity
Coated ELISA plate	12-Well * 8Tubes	Washing concentrate (30X)	20ml
Standard dilution	3ml	Instruction	1
Chromogen solution A	6ml	Seal plate membrane	2
Chromogen solution B	6ml	Hermetic bag	1
Streptavidin-HRP	6ml	Stop solution	6ml
Standard solution (400μmol/L)	0.5ml	Anti SDMA antibodies labeled with biotin	1ml

MATERIALS REQUIRED BUT NOT SUPPLIED

1. An incubator which can provide stable incubation conditions up to 37°C±0.5°C. .
2. Microplate reader with 450 ± 10nm filter.
3. Precision pipettes and Disposable pipette tips.
4. Distilled water.
5. Deionized or distilled water.
6. Absorbent paper.

STORAGE OF THE KITS

1. For unopened kit: All the reagents should be kept according to the labels on vials. The TMB Substrate Wash Buffer (30 × concentrate) and Stop Solution should be stored at 4°C upon receipt while the others should be at -20°C.
2. For opened kit: When the kit is opened, the remaining reagents still need to be stored according to the above storage condition.

Note： It is highly recommended to use the remaining reagents within 1 month provided this is within the expiration date of the kit. For the expiration date of the kit, please refer to the label on the kit box. All components are stable until this expiration date.

IMPORTANT NOTES

1. Before opening the kit kept at the temperature of 2-8℃, it takes at least 30 minutes to increase naturally to room temperature. After breaking the seal of ELISA coated-plate, some of the stripes used should be kept in hermetic bag.

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 ELISA reader must be set as the standard of determining the experiment result.

4.Pipette tips and seal plate membrane in hand should not be used more than once in order to avoid cross contamination.

5.All samples, washing concentration and wastes of every kind should be disposed as infective agent.

6.Other reagents not needed must be packed or covered. Reagents of different batches must not be mixed and should be used before their respective validity dates.

7. Substrate B is sensitive to light and therefore should not be exposed to light for too long.

SWASHING METHODC

Manually washing method: Washing by hand: Shake off the liquids in the wells of the ELISA plate; Lay several bibulous papers on the test bed and pat hard the ELISA plate several times downward; then inject at least 0.35ml of diluted washing concentration for 1-2 minutes' soaking. Repeat this process as needed.

Automatic washing method: Washing by automatic plate washer: If there is an automatic plate

washer, it should only be used in the test when you are quite familiar with its functions.

SAMPLE COLLECTION AND STORAGE

- Samples containing NaN3 must not be tested as it inhibits the activity of Horse Radish Peroxidase (HRP).
- 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℃. Avoid repeated freeze-thaw cycles.
- Serum: Allow the serum to clot for 10-20 minutes at room temperature. Centrifuge (at 2000-3000 RPM) for 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.
- Blood 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 10-20 minutes. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.
- Urine: Collect by sterile tube. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again. When collecting pleuroperitoneal fluid and cerebrospinal fluid, please follow the procedures above-mentioned.
- Cell culture supernatant: Collect by sterile tubes when examining secrete components. Centrifuge (at 2000-3000 RPM) for approximately 20 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 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.
- Tissue sample: Incise sample and weigh up. Add a certain amount of PBS (PH 7.4). Freeze with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8℃. Add a certain amount of PBS (PH 7.4) and then homogenize the sample thoroughly by hand or homogenizer. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. Aliquot and keep one for examination and freeze the others for later use.

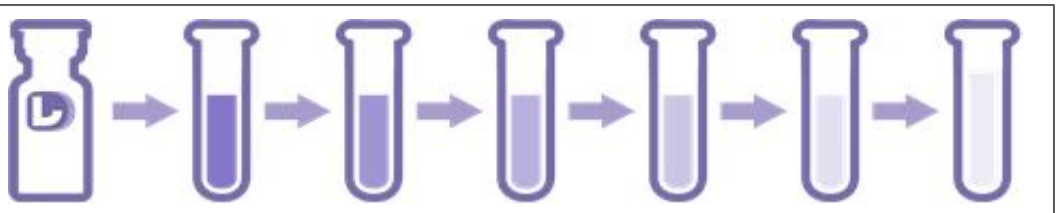
Note:

1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen can not be detected.
3. When performing the assay, bring samples to room temperature.

ASSAY PROCEDURE

1.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.):

200 μ mol/L	Standard No.5	120μl Original Standard + 120μl Standard diluents
100 μ mol/L	Standard No.4	120μl Standard No.5 + 120μl Standard diluents
50 μ mol/L	Standard No.3	120μl Standard No.4 + 120μl Standard diluent
25 μ mol/L	Standard No.2	120μl Standard No.3 + 120μl Standard diluent
12.5 μ mol/L	Standard No.1	120μl Standard No.2 + 120μl Standard diluent

Stock standard						
Tube	**standard**	**S5**	**S4**	**S3**	**S2**	**S1**
μ mol/L	400	200	100	50	25	12.5

2.The number of stripes needed is determined by that of samples to be tested added by that of standards. It is suggested that each standard solution and each blank well should be arranged with three or more wells as much as possible.

3.Sample injection: 1) Blank well: Add only Chromogen solution A and B, and stop solution.

2) Standard solution well: Add 50μl standard and streptavidin-HRP 50μl. 3) Sample well to be tested: Add 40μl sample and then 10μl SDMA antibodies, 50μl streptavidin-HRP. Then cover it with seal plate membrane. Shake gently to mix them up. Incubate at 37℃ for 60 minutes.

4.Preparation of washing solution: Dilute the washing concentration (30X) with distilled water for later use.

5.Washing: Remove the seal plate membrane carefully, drain the liquid and shake off the remaining liquid. Fill each well with washing solution. Drain the liquid after 30 seconds' standing. Then repeat this procedure five times and blot the plate.

6. Color development: Add 50μl chromogen solution A firstly to each well and then add 50μl chromogen solution B to each well as well. Shake gently to mix them up. Incubate for 10 minutes at 37℃ away from light for color development.

7.Stop: Add 50μl Stop Solution to each well to stop the reaction (the blue color changes into yellow immediately at that moment).

8.Assay: Take blank well as zero, measure the absorbance (OD) of each well one by one under 450nm wavelength, which should be carried out within the 10 minutes after having added the stop solution.

9.According to standards' concentrations and the corresponding OD values, calculate the linear regression equation of the standard curve. Then according to the OD value of samples, calculate the concentration of the corresponding sample. Special software could be employed to calculate as well.

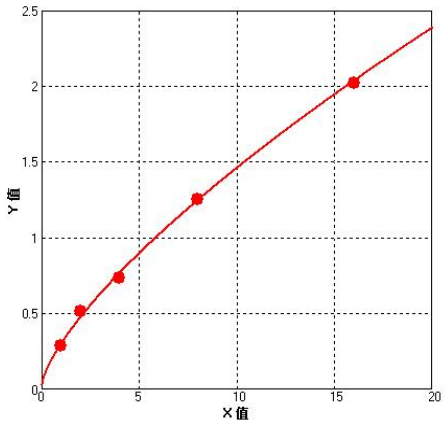
TEST PRINCIPLE

This kit uses enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the Mouse symmetric dimethylarginine (SDMA). Add symmetric dimethylarginine(SDMA)to the wells, which are pre-coated with symmetric dimethylarginine(SDMA)monoclonal antibody and then incubate. After that, add anti SDMA antibodies labeled with biotin to unite with streptavidin-HRP, which forms immune complex. Remove unbound enzymes after incubation and washing. Add substrate A and B. Then the solution will turn blue and change into yellow with the effect of acid. The shades of solution and the concentration of Mouse symmetric dimethylarginine (SDMA) are positively correlated.

CALCULATION OF RESULTS

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.



Note： **THE CHART IS FOR REFERENCE ONLY AND THE ACTUAL PERFORMANCE DON'T HAVE TO BE THE SAME AS THE CHART**

SPECIFICITY

This assay has high sensitivity and excellent specificity for detection of SDMA.

No significant cross-reactivity or interference between SDMA and analogues was observed.

Note： Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between SDMA and all the analogues, therefore, cross reaction may still exist.

PRECISION

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level SDMA were tested 20 times on one plate, respectively.

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

CV(%) = SD/meanX100

Intra-Assay: CV<8%

Inter-Assay: CV<10%

STABILITY

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5% within the expiration date under appropriate storage condition.

Note: To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards;
2. Add prepared samples, standards and ELISA solutions. Let them react for 60 minutes at 37 ℃.
3. Wash the plate five times. Add Chromogen solution A and B. Incubate for 10 minutes at 37 ℃; for color development.
4. Add stop solution
5. Read the OD value within 10minutes.
6. Calculate.

ASSAY RANGE: 3μmol/L→380μmol/L

SENSITIVITY: 1.43μmol/L

IMPORTANT NOTE

1. Limited by the current condition and scientific technology, we can't completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit.
2. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available.
3. Kits from different batches may be a little different in detection range, sensitivity and color developing time. Please perform the experiment exactly according to the instruction attached in kit while electronic ones from our website is only for information.
4. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
5. Protect all reagents from strong light during storage and incubation. All the bottle caps of

reagents should be covered tightly to prevent the evaporation and contamination of microorganism.

6. There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microtiter plate from the storage bag until needed.
7. Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at 450 ± 10nm wavelength is acceptable for use in absorbance measurement. Please read the instruction carefully and adjust the instrument prior to the experiment.
8. Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended.
9. Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too.
10. Kits from different manufacturers with the same item might produce different results, since we haven't compared our products with other manufacturers.
11. Valid period: 12 months.
12. The instruction manual also suits for the kit of 48T, but all reagents of 48T kit are reduced by half.

PRECAUTION

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material