

General Asymmetrical Dimethylarginine ELISA Kit (ADMA)

Catalog NO.: RK04233

version: 2.0

This package insert must be read in its entirety before using this product

Introduction

This kit uses the competitive method to quantitatively determine the content of ADMA in serum, plasma, tissue homogenate, cell lysate, cell culture supernatant or other biological body fluids.

Principle of the Assay

This assay employs the competitive inhibition enzyme immunoassay technique. An antibody specific to ADMA has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotin labeled ADMA and unlabeled ADMA (Standards or samples) with the pre-coated antibody specific to ADMA. After incubation the unbound conjugate is washed off. Next, avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of ADMA in the sample. After addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of ADMA in the sample.

Material Provided & Storage Conditions

Store unopened kit at 2-8 °C. Do not use past kit expiration date. It is highly recommended to use the remaining reagents within 1 month provided this is prior to the expiration date of the kit.

| Part | Size | Cat. No. | Storage of opened/reconstituted material |
|--|----------|----------|--|
| Antibody Coated Plate | 8×12 | RM94416 | Return unused wells to the foil pouch containing the desiccant pack and store at ≤ -20 °C. Reseal along entire edge of zip-seal. |
| Standard Lyophilized | 2 | RM94417 | Aliquot and store at ≤ -20 °C in a manual defrost freezer. Avoid repeated freeze-thaw cycles. |
| Concentrated Biotin- Conjugate Antigen(100×) | 1 ×60μL | RM94418 | May be stored for up to 6 month at -20 °C.* |
| Streptavidin-HRP Concentrated (100×) | 1 ×120ul | RM94419 | |
| Standard/Sample Diluent (R1) | 1 ×20mL | RM00023 | May be stored for up to 6 month at 2-8 °C.* |
| Biotin- Conjugate Antigen Diluent(R2) | 1 ×10mL | RM00024 | |
| Streptavidin-HRP Diluent(R3) | 1 × 10mL | RM00025 | |
| Wash Buffer(25x) | 1 ×30mL | RM00026 | |
| TMB Substrate | 1 ×10mL | RM00027 | |
| Stop Solution | 1 ×10mL | RM00028 | |
| Plate Sealers | | | 4 Strips |
| Specification | | | 1 |

Other Supplies Required

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm or 570 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. Incubator.
6. Test tubes for dilution of standards and samples.

Precautions

1. FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
2. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
3. Variations in sample collection, processing, and storage may cause sample value differences.
4. Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.
5. Stop Solution contains strong acid. Wear eye, hand, and face protection.
6. Apart from the standard of kits, other components should not be refrigerated.
7. Please perform simple centrifugation to collect the liquid before use.
8. Do not mix or substitute reagents with those from other lots or other sources.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the

lowest frequency.

10. Mix the sample and all components in the kits adequately, and use clean plastic container to prepare all of the diluent.
11. Both the sample and standard should be assayed in duplicate, and the sequence of the reagents should be added consistently.
12. Reuse of dissolved standard is not recommended.
13. The kit should not be used beyond the expiration date on the kit label.
14. The kit should be away from light when it is stored or incubated.
15. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
16. To avoid cross contamination, please use disposable pipette tips.
17. Please prepare all the kit components according to the Specification. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.
18. This assay is designed to eliminate interference by other factors present in biological samples.
19. Until all factors have been tested in this assay, the possibility of interference cannot be excluded.
20. The 48T kit is also suitable for the specification.

Sample Collection & Storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Samples containing the correlated IgG as in this kit may interfere with this assay.

Cell Culture Supernates : Remove particulates by centrifugation. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Serum : Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at $1000 \times g$. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma : Collect plasma using EDTA or Heparin as an anticoagulant. Centrifuge for 15 minutes at $1000 \times g$ within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles. (Note: Citrate plasma has not been validated for use in this assay.)

Tissue homogenates : The preparation of tissue homogenates will vary depending upon tissue type. Tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed before homogenization. Minced the tissues to small pieces and homogenized them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular location of the target protein) (e.g. 1mL lysis buffer is added in 200mg tissue sample) with a glass homogenizer on ice (Micro Tissue Grinders works, too). The resulting suspension was sonicated with an ultrasonic cell disrupter till the solution is clarified. Then, the homogenates were centrifuged for 5

minutes at 10,000xg. Collect the supernates and assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$.

Other biological fluids: Centrifuge samples for 20 minutes at 1,000xg. Collect the supernates and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

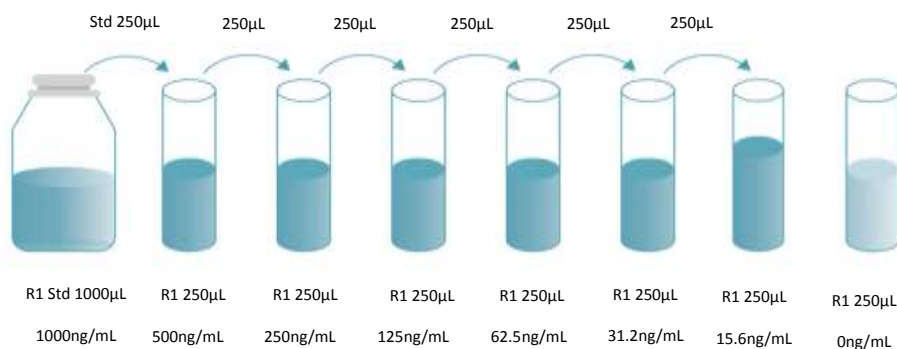
Note : It is suggested that all samples in a study be collected at the same time of the day. Avoid hemolytic and hyperlipidemia sample for Serum and Plasma.

Reagent Preparation

Bring all reagents to room temperature before use. If crystals have formed in the concentrate, Bring the reagent to room temperature and mix gently until the crystals have completely dissolved.

Standard - Reconstitute the Standard Lyophilized with 1.0 mL Standard/Sample Diluent(R1). This reconstitution produces a stock solution of 1000 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use the 1000 ng/mL standard stock to produce a dilution series (below) with Standard/Sample Diluent(R1). Mix each tube thoroughly and change pipette tips between each transfer (recommended concentration for standard curve: 1000, 500, 250, 125, 62.5, 31.2, 15.6, 0 ng/mL). Use diluted standards within 60 minutes of preparation.



Working Biotin Conjugate Antibody - Dilute 1:100 of Concentrated Biotin

Conjugate Antibody (100x) with Biotin-Conjugate Antibody Diluent (R2) before use, for example: Add 20 μL of Concentrated Biotin Conjugate Antibody (100x) to 1980 μL Biotin-Conjugate Antibody Diluent (R2) to prepare 2000 μL Working Biotin Conjugate Antibody Buffer.

Working Streptavidin-HRP - Dilute 1:100 of Concentrated Streptavidin-HRP (100x) with Streptavidin-HRP Diluent (R3) before use, for example: Add 20 μL of Concentrated Streptavidin-HRP (100x) to 1980 μL Streptavidin-HRP Diluent (R3) to prepare 2000 μL Working Streptavidin-HRP Buffer.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 1:25 with double distilled or deionized water before use, for example : Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Prepare the Biotin Conjugate Antigen Concentrated (100X) Working Solution 15 minutes early before use.
3. Add 50 μ L Standard/sample Diluent (R1) in blank well, add 50 μ L different concentration of standard and sample in other wells. And then add Biotin Conjugate Antigen Working Solution in each wells (50 μ L/well), cover with new adhesive strip provided. Incubate for 1 hour at 37°C.
4. Prepare the Streptavidin-HRP Concentrated (100X) Working Solution 15minutes early before use.
5. Remove the liquid of each well, Add wash buffer 350 μ L/well, aspirate each well after holding 60-120 seconds, repeating the process two times for a total of three washes.
6. Add Streptavidin-HRP Working Solution in each wells (100 μ L/well), cover with new adhesive strip provided. Incubate for 30 minutes at 37°C.
7. Warm-up the Microplate reader.
8. Remove the liquid of each well, Add wash buffer 350 μ L/well, aspirate each well after holding 60-120 seconds, repeating the process four times for a total of five washes.
9. Add TMB Substrate (90 μ L/well). Incubate for 15-20 minutes at 37°C .Protect

from light.

10. Add Stop Solution (50 μ L/well), determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Assay Procedure Summary

Prepare the standard and reagents



Add 50ul of standards and test samples to each well



Add 50ul Working Biotin-Conjugate Antigen



Incubate for 1 hours at 37°C, then wash 3 times



Add 100ul Working Streptavidin-HRP



Incubate for 0.5 hour at 37°C, then wash 5 times



Add 90ul Substrate Solution



Incubate for 15-20 min at 37°C under dark condition



Add 50ul Stop Solution



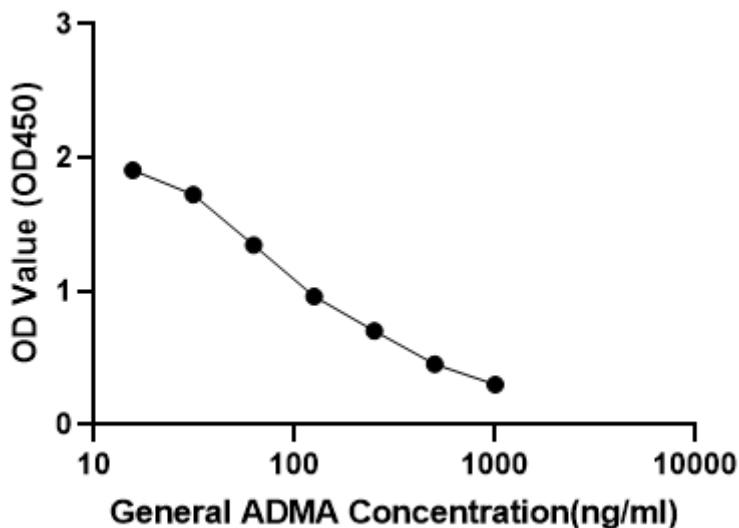
Detect the optical density within 5 minutes under 450nm.

Correction Wavelength set at 570nm or 630nm

Calculation of Results

1. Average the duplicate readings for each standard, control and sample, and subtract the average zero standard optical density (O.D.).
2. 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 on the X-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the ADMA concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data



The standard curves are provided for demonstration only. A standard curve should be generated for each set of ADMA assayed.

Sensitivity

The minimum detectable dose (MDD) of ADMA typically less than 5.4 ng/mL. The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

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

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

Note:

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

Precision

Intra-plate Precision

3 samples with low, middle and high level ADMA were tested 20 times on one plate, respectively.

Intra-Assay: CV<10%

Inter-plate Precision

3 samples with low, middle and high level ADMA were tested on 3 different plates, 8 replicates in each plate.

Inter-Assay: CV<15%

Recovery

Matrices listed below were spiked with certain level of ADMA and the recovery rates were calculated by comparing the measured value to the expected amount of ADMA in samples.

| Sample | Average Recovery (%) | Range (%) |
|-------------------------|----------------------|-----------|
| Cell Culture Media(n=5) | 91 | 83-103 |
| Serum(n=5) | 87 | 80-101 |

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of ADMA and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

| / | / | Cell Culture Media(n=5) | Serum(n=5) |
|------|-------------------------|-------------------------|------------|
| 1:2 | Average of Expected (%) | 89 | 95 |
| | Range (%) | 85-120 | 80-116 |
| 1:4 | Average of Expected (%) | 112 | 85 |
| | Range (%) | 92-118 | 80-97 |
| 1:8 | Average of Expected (%) | 115 | 89 |
| | Range (%) | 95-120 | 80-102 |
| 1:16 | Average of Expected (%) | 113 | 83 |
| | Range (%) | 90-119 | 80-86 |

Trouble Shooting

| Problem | Possible Cause | Solution |
|---------|----------------|----------|
|---------|----------------|----------|

| | | |
|--------------------------|--|--|
| High Background | Insufficient washing | Sufficiently wash plates as required. Ensure appropriate duration and number of washes. Ensure appropriate volume of wash buffer in each well. |
| | Incorrect incubation procedure | Check whether the duration and temperature of incubation are set up as required. |
| | Cross-contamination of samples and reagents | Be careful of the operations that could cause cross-contamination. Use fresh reagents and repeat the tests. |
| No signal or weak signal | Incorrect use of reagents | Check the concentration and dilution ratio of reagents. Make sure to use reagents in proper order. |
| | Incorrect use of microplate reader | Warm the reader up before use. Make sure to set up appropriate main wavelength and correction wavelength. |
| | Insufficient colour reaction time | Optimum duration of colour reaction should be limited to 15-25 minutes. |
| | Read too late after stopping the colour reaction | Read the plate in 5 minutes after stopping the reaction. |
| | Matrix effect of samples | Use positive control. |
| Too much signal | Contamination of TMB substrate | Check if TMB substrate solution turns blue. Use new TMB substrate solution. |
| | Plate sealers reused | Use a fresh new sealer in each step of experiments. |
| | Protein concentration in sample is too high | Do pre-test and dilute samples in optimum dilution ratio. |
| Poor Duplicates | Uneven addition of samples | Check the pipette. Periodically calibrate the pipette. |
| | Impurities and precipitates in samples | Centrifuge samples before use. |
| | Inadequate mixing of reagents | Mix all samples and reagents well before loading. |

*For research purposes only. Not for therapeutic or diagnostic purposes.