

Direct Symmetric Dimethylarginine (SDMA) ELISA

For Research Use Only

Cat. No.: BW-SDMA ELISA

1. Introduction

Symmetric dimethylarginine (SDMA) is a small-molecule compound produced via arginine methylation, which is released into the bloodstream during protein breakdown. SDMA is mainly cleared through renal excretion and serves as a valuable endogenous biomarker for evaluating glomerular filtration rate (GFR) [1].

In veterinary diagnostics, SDMA correlates well with glomerular filtration rate (GFR) in small animals, such as dogs and cats. SDMA increases earlier than creatinine (CREA), and so it is more sensitive for diagnosis of both active and chronic kidney disease. Unlike CREA, SDMA is not impacted by lean body mass. The SDMA Test now become a parameter for early detection of kidney diseases of small animals [2, 3, 4].

This kit is used for direct quantification of SDMA concentrations in biological samples.

2. Principle of the Assay

The SDMA ELISA adopts a typical competitive immunoassay principle.

SDMA present in samples, calibrators, and controls competes with SDMA coated on the microplate wells for binding a limited number of anti-SDMA monoclonal antibodies. Unbound components are removed by washing. Horseradish peroxidase (HRP)- conjugated secondary antibody is added to trace the bound primary antibody. After a final wash, TMB substrate is added to initiate a color reaction. The absorbance intensity is inversely proportional to the SDMA concentration in the sample. A calibration curve is established using calibrators to calculate the SDMA concentrations in unknown samples.

3. Package Contents

Kit contents are sufficient for **96 tests** (8×12 wells).

Expiry date is labeled on the outer box.

Components	Specification	Description
① SDMA Coated Plate	8×12 wells, detachable	Microwells pre-coated with SDMA antigen
② Primary Antibody	1 bottle (0.5mL, 25×)	Monoclonal antibody against SDMA
③ Assay Buffer	1 bottle (30mL)	PBS buffer containing BSA and Tween-20
④ Secondary Antibody	1 bottle (0.5mL, 25×)	Sheep anti-mouse IgG- HRP conjugate
⑤ TMB Chromogenic Substrate	1 bottle (15mL)	Single- component TMB for color development
⑥ Wash Buffer	2 bottles (25mL/bottle, 10×)	0.05% Tween-20 in PBS
⑦ Stop Solution	1 bottle (15mL)	1 M Citric acid solution
⑧ SDMA Calibrators	6 vials (1mL each)	0, 62.5, 125, 250, 500, 1000ng/mL

4. Kit Storage and Stability

- 1) Store the entire kit at **2–8°C**.
- 2) Unused plate strips must be kept in the aluminum foil bag with desiccant; keep sealed and dry.
- 3) Shelf life: **12 months** from production.
- 4) Use opened reagents and plate strips within **3 months**.

5. Equipment and Materials Required but not Provided

- 1) Single-channel micropipettes (10–100 μ L) and disposable tips
- 2) 8-/12-channel micropipette (50–200 μ L)
- 3) Orbital microplate shaker
- 4) Microplate reader (detection wavelength: 450nm)
- 5) Distilled or deionized water
- 6) Microplate washer (optional)

6. Sample Collection and Storage

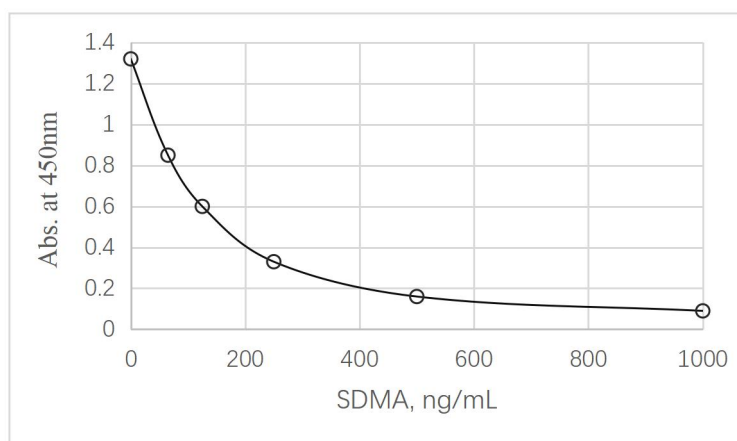
- 1) The test can be performed with serum and plasma as samples. The applicability for testing samples other than plasma or serum remains to be verified.
- 2) Hemolytic and lipemic samples should not be used.
- 3) The samples can be stored up to one month at 2 - 8°C. For a longer storage (up to 18 months) the samples need to be frozen at -20°C. Repeated freezing and thawing should be avoided.
- 4) Hemolytic, lipemic and icteric specimens should not be used.

7. Experimental Procedure

- 1) Separate the strips to be used by cutting through the protective tape with a sharp knife and transfer them to a strip frame. Pull off the protective tape from the strips intended for use. (Return the remaining strips to the plastic tray pack and reseal)
- 2) According to the number of samples to be tested, add the Primary Antibody to the required volume of Assay Buffer at a ratio of 1:25, and mix thoroughly. This should be done within 60 minutes before use.
- 3) Pipette 25 μ L of the SDMA calibrators (0, 62.5, 125, 250, 500, 1000ng/mL) and samples into the bottom of each well.
- 4) Add 75 μ L of diluted Primary Antibody solution to each well, incubate the frame for 90 minutes at room temperature on an orbital shaker at medium speed.
- 5) Discard or aspirate the contents of the wells and wash thoroughly with 300 μ L wash buffer diluted 10 times with distilled water, remove residual liquid by tapping the inverted plate on a clean absorbent paper. Repeat this 3 times. Preferably, a Microplate Washer may be used.
- 6) Within 60 minutes before use, prepare the needed volume of Secondary Antibody solution by mixing 1.5mL Assay Buffer with 60 μ L Secondary Antibody stock solution (25 $\times$) per strip.
- 7) Dispense 100 μ L of diluted Secondary Antibody solution into each well and incubate the frame for 60 minutes at room temperature on an orbital shaker at medium speed.
- 8) After incubation, wash the wells as in step 7-5). Ensure no residual wash buffer remains.
- 9) Dispense 100 μ L of Chromogenic Substrate solution into each well, and mix thoroughly. Incubate for 20 minutes at room temperature with slow shaking.
- 10) Add 100 μ L of Stop Solution into each well. Shake for at least 10 seconds on an orbital shaker.
- 11) Quantitation. Read the optical density at 450nm in a microplate photometer within 10 minutes. Plot absorbance vs. calibrator concentration to construct the SDMA calibrator curve. When using analyzing software, it is recommended to use the Four Parameter Logistic (4PL) Regression. The concentration of the controls and samples in ng/ml can be read directly from this calibration curve by using their average optical density.

7. Performance Characteristics

- 1) The typical standard curve for the SDMA ELISA is shown below.



- 2) Analytical sensitivity. The analytical sensitivity of the SDMA ELISA is typically better than 12ng/mL, if defined as the mean of the measurement values for the zero calibrator minus 2 standard deviations (mean value - 2SD).
- 3) Recovery. Spiked serum samples were prepared by adding varying levels of SDMA to pooled serum specimens containing a known amount of SDMA. Recoveries were in the range of 96 - 117% with a mean value of 105.2% (n=15).
- 4) Cross reactivity (at the 50% displacement level)

Substance	Cross Reactivity (%)
SDMA	100
ADMA	0.22%
N ^G -Monomethyl-L-arginine (MMA)	0.86%
Homoarginine	<0.01%
Creatinine	<0.01%
Arginine	<0.01%

- 5) Intra-assay Precision: $\leq 10\%$, Inter-assay Precision: $\leq 15\%$.
- 6) The range of measurement: 100 - 1600ng/mL.

8. Notes

- 1) This kit is for research use only, not for human diagnostic or therapeutic procedures.
- 2) Avoid repeated freezing and thawing of reagents.
- 3) Protect TMB substrate from light and avoid contact with metal ions.
- 4) Strictly follow incubation time and temperature to ensure reproducibility.
- 5) If the OD value of the sample exceeds the range of the calibration curve, the sample needs to be diluted with the zero-concentration calibrator for detection to ensure that the result is within the linear range.

9. References

- 1) Hendrik Meyer, Marcus Dörr, Anke Hannemann, et al. Symmetric dimethylarginine as a biomarker of renal impairment after a decade of follow-up. *Scientific Reports*, 2025, 15, 30520.
- 2) Rebecca Kohnken, Lauren Himmel, Michael Logan, et al. Symmetric Dimethylarginine is a Sensitive Biomarker of Glomerular Injury in Rats. *Toxicologic Pathology*. 2022, 50, 176-185.
- 3) Samantha CL, James MT , Timothy LW. Evaluation of symmetric dimethylarginine in cats with acute kidney injury and chronic kidney disease. *Journal of Veterinary Internal Medicine*, 2022, 36, 1669–1676.
- 4) Portia Tshidi Mashego, Emma H. Hooijberg. Analytical and Quality Control Validation of a Novel Symmetric Dimethylarginine Assay in Dogs and Cats. *Veterinary Clinical Pathology*, 2025, 54, 239–250.

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