

**KAMIYA BIOMEDICAL COMPANY**

# Urinary Diacetylspermine ELISA Kit

**For the quantitative determination of urinary diacetylspermine in rodents.**

**Cat. No. KT-617**

**For Research Use Only.**

**PRODUCT INFORMATION****Urinary Diacetylspermine ELISA Kit**  
Cat. No. KT-617**INTENDED USE**

The Urinary Diacetylspermine EIA is for the quantitative determination of urinary diacetylspermine in rodents. For research use only.

**INTRODUCTION**

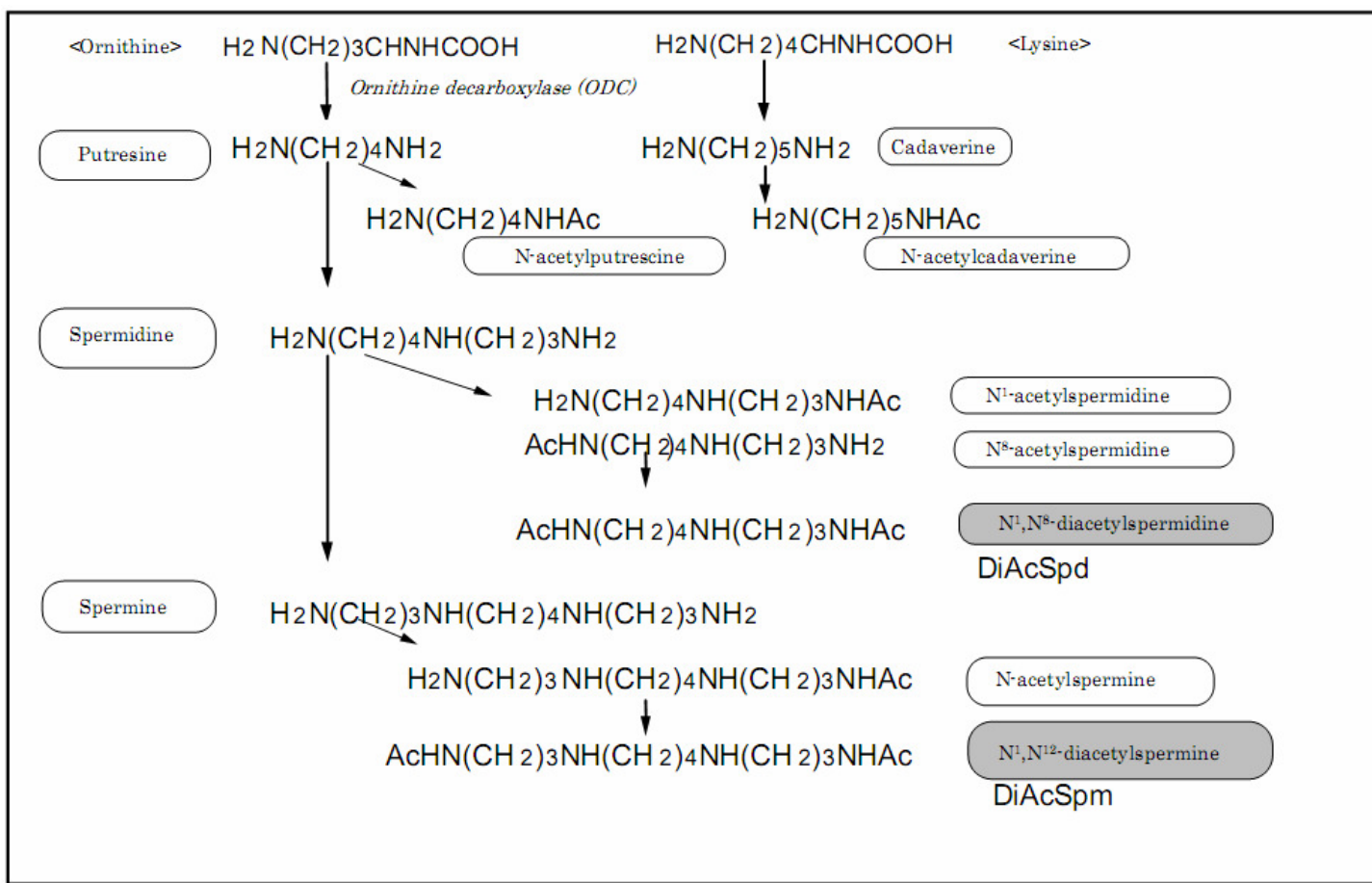
Polyamines are generally believed to function both in protein synthesis and DNA synthesis leading to control cell proliferation. It is reported that the total amount of urinary polyamines are elevated in cancer patients. Assays have already been developed to measure the total amount of these urinary polyamines.

Recently two diacetyl-derivatives, N<sup>1</sup>, N<sup>12</sup>-diacetylspermine and N<sup>1</sup>, N<sup>8</sup>-diacetylspermine, were found to correlate to the status of disease more closely than the total amount of polyamines. Our kit is convenient for use quantifying the amount of urinary diacetylspermine by using the ELSA method. This kit is for research use only.

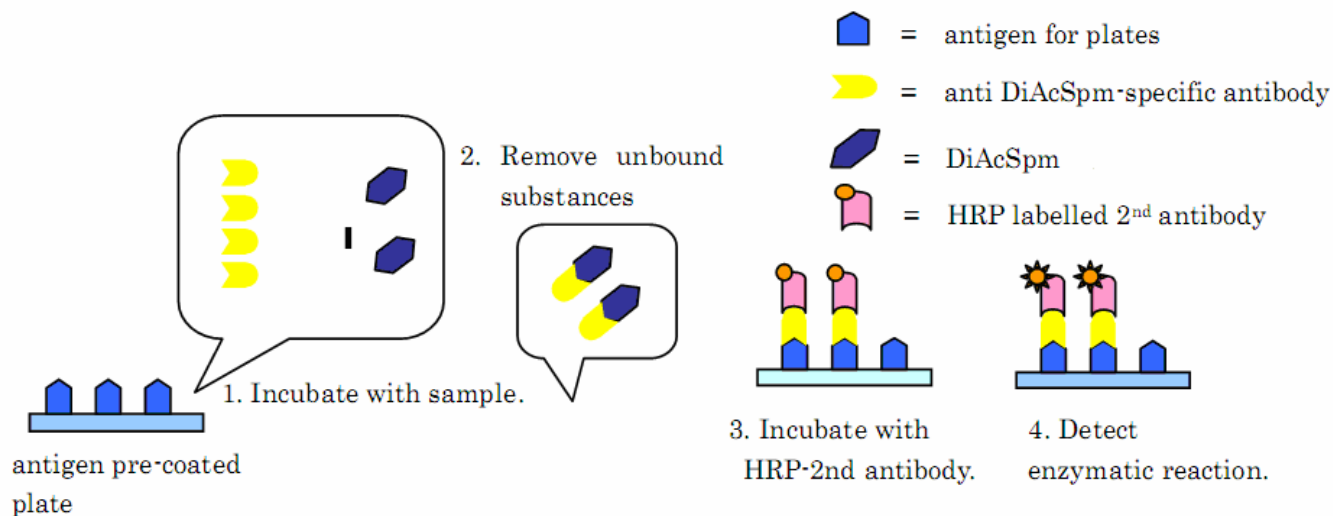
Highly sensitive and specific

Strip type well, antigen pre-coated microplate

Assay range 6.25-200 nM



## [measurement principle]

**COMPONENTS**

| Reagent                         | Volume          | Concentration    |
|---------------------------------|-----------------|------------------|
| Wash Solution                   | 30 mL           | 20x concentrate  |
| Antibody Diluent                | 20 mL           |                  |
| Antibody Plate                  | 96 wells        | 1 plate          |
| Calibrator                      | 2 x 250 $\mu$ L |                  |
| Anti-Diacetylspermine Antibody  | 60 $\mu$ L      | 100x concentrate |
| HRP-Anti Rabbit IgG Antibody    | 80 $\mu$ L      | 80x concentrate  |
| OPD (o-phenylendiamine) tablets | x2              |                  |
| Substrate Solution              | 30 mL           |                  |
| Stop Solution                   | 15 mL           |                  |
| Dilution Plate                  | 1 plate         |                  |

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Micropipette and tip
- Plate washer
- Plate reader

**PREPARATION**

**Wash Buffer-** Make sure that wash buffer concentrate does not contain any crystallized material prior to use. Working solution is prepared by dilution of 30 mL of wash buffer concentrate with 570 mL of distilled or deionized water. For convenience this solution can be kept at 4 °C for up to 14 days.

**Sample Diluent-** Mix whole volume (50 mL) of the stock sample diluent with 200 mL of deionized water. Store solution at 4 °C after preparation.

Calibrator- Prepare 6 calibrators by serial dilution of diacetylspermine calibrator concentrate (200 nM) as follows:

We recommend using a polypropylene tube for preparation of calibrator solution. A glass or polystyrene tube may cause non-specific adsorption of diacetylspermine, so that you may not get reliable results.

|            | 200 | 100 | 50.0 | 25.0 | 12.5 | 6.25 (nM) |
|------------|-----|-----|------|------|------|-----------|
| 200nM (μL) | 250 | 100 | 100  | 100  | 100  | 100       |
| (μL)       | 0   | 100 | 100  | 100  | 100  | 100       |

Anti-Diacetylspermine Antibody- Mix 40 μL of the anti-diacetylspermine antibody stock solution with 4 mL of the diluent solution for the 96 well reaction. Diluted antibody should not be stored.

HRP-Anti Rabbit IgG Antibody- Dilute 65 μL HRP-antib rabbit IgG antibody concentrate with 5.2 mL of diluent solution for 96 well reaction. Diluted antibody should not be stored.

Coloring Solution- Add one OPD tablet to 13 mL of substrate buffer to reconstitute the coloring solution just before use. This solution should not be stored.

## ASSAY PROCEDURE

### Sample preparation

1. Collect urine in sampling tube. Add 0.1% Na<sub>2</sub>N<sub>3</sub> at final concentration.
2. After centrifugation at 1500 rpm for 5 minutes, dilute the resulting supernatant over 4 times with distilled water.
3. Measure the amount of creatinine in the remaining diluted supernatant for compensation.
4. Prepared urine should be kept below -30 °C if necessary.

### Procedure

1. Pre-reaction- Prepare the calibrator control wells containing 70 μL of anti-Diacetylspermine antibody solution and 70 μL of the 6 calibrators (200, 100, 50, 25, 12.5, 6.25 nM) in the dilution plate. Likewise prepare the experimental wells containing 70 μL of anti-Diacetylspermine antibody solution and 70 μL of prepared urinary sample in the same plate. After settlement, incubate at room temperature for 1 hour. \*Above reaction volumes can be applied for double measurements of primary reaction. If a single measurement reduce to 40 μL of each solution.
2. Preparation of reaction plate- Add 300 μL of wash solution to each well and wait another 30 minutes. Discard the wash solution from the wells completely and wash with 300 μL of wash solution. Wash in this manner 2 more times.
3. Primary reaction- Apply 50 μL/well x2 (in the case of measuring double wells) pre-reaction solutions and incubate for 1 hour. After incubation discard the reaction solution and wash with 300 μL of wash solution. Repeat this step another 2 times.
4. Secondary reaction- Apply 50 μL of HRP-anti rabbit IgG antibody and incubate for 1 hour. Equilibrate substrate buffer to room temperature prior to use. After incubation, discard the reaction solution and wash with 300 μL of wash solution. Repeat this step another 2 times.
5. Coloring- Apply 100 μL of coloring solution to each well and incubate for 10 minutes at room temperature.
6. Stop reaction- Apply 100 μL of stop solution to stop the enzymatic reaction.
7. Read absorbances at 490 nm or 492 nm with a microplate reader.
8. Determine the concentration in samples using the calibration curve.

## RESULTS

In general, the concentration of urine changes easily by the amount of water taken in or by the environment. The amount of urinary creatinine depends on the amount of muscle and their measurements correlate positively. Therefore, the correct measurement of Diacetylspermine concentration in urine with creatinine concentration as follows:

$$\text{Data correction} : \text{nmol/g} \cdot \text{cre} = \frac{\text{Diacetylspermine concentration (nM)}}{\text{Creatinine concentration (mg/dL)}} \times 100$$

**[Reproducibility]**

Domain of standard curve : 6.25~200nM

Minimum measurement range for detection: 12.5nM

Minimum dilution number of urine sample : × 4

Minimum sensitivity for detection: 50.0nM

Within-run (n=20, 2 concentration): CV (%) = 4.87, 5.20

Between-run (n=20, 2 concentration): CV (%) = 7.98, 9.50

Recovery test: In the recovery study, recoveries 99.8% and 98.2%, 108%, 100% were obtained for 2, 4, 8times dilutions of the sample urine

Coexistence substance : No influence to Hemoglobin 400mg/dL · Bilirubin 10mg/dL ·  
Glucose 1000mg/dL · Ascorbic acid 100mg/dL

Comparison between the ELISA kit and HPLC values  $Y = 1.01X + 73.2$   $R^2=0.978$

**Storage**

All reagents should be stored at -30°C.

**FOR RESEARCH USE ONLY****KAMIYA BIOMEDICAL COMPANY**

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