

Urinary Diacetylspermine ELISA Kit

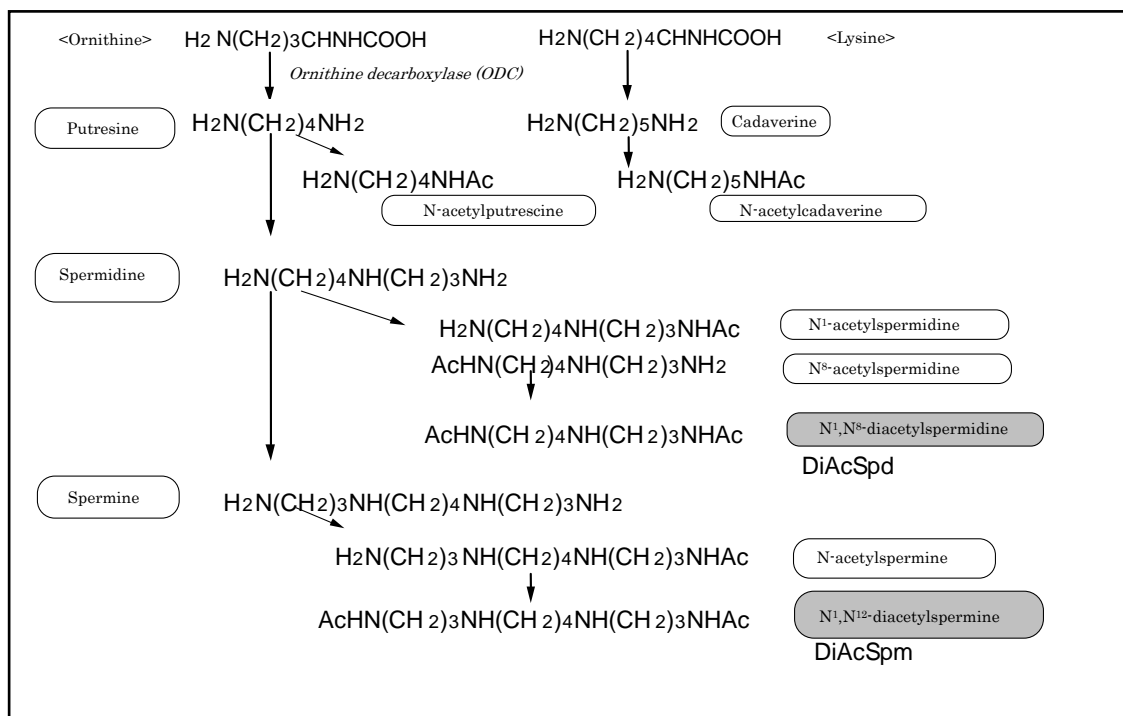
Polyamines are generally believed to function both in protein synthesis and DNA synthesis leading to control cell proliferation. In 1971, Russel firstly reported that total amount of urinary polyamines elevated in cancer patients. And quantitative kit of urinary polyamines were already developed and utilized as a general biochemical examination.

Recently two diacetyl-derivatives, N1, N12-diacetylspermine and N1, N8-diacetylspermidine, were found to be excreted in urine and form 0.6% and 1.4% of total polyamines respectively.

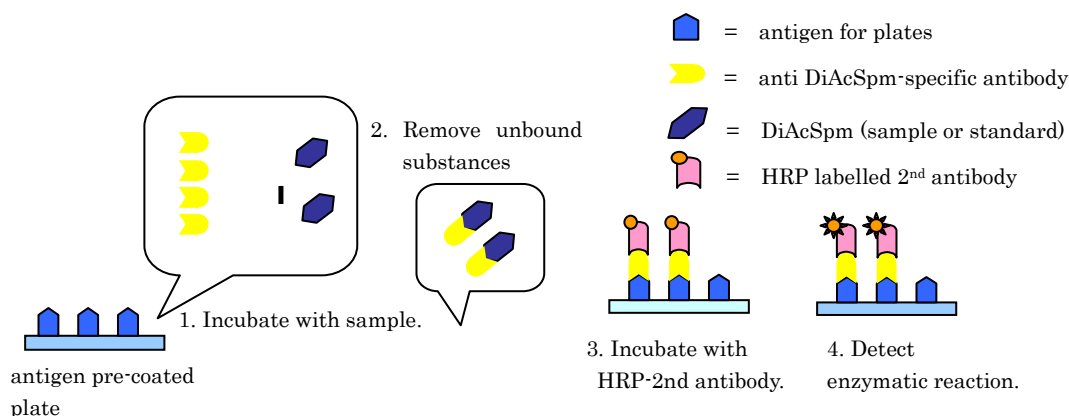
Comparing urine of diseased person with urine of healthy person, some reports suggested the possibility that diacetyl-derivatives correlate to the status of disease more closely than total amount of polyamines.

Our kit is convenient to quantify amount of urinary diacetylspermine by using ELISA method. This kit is only for research use, not for diagnosis.

- Highly sensitive and specific
- Strip type well, antigen pre-coated microplate
- Assay range: 6.25~200nM



[measurement principle]



[Kit Contents]

- | | |
|---|------------------------|
| (1) Antigen coated microtiter plate,96 wells | 1 plate |
| (2) Diacetylspermine standard | 250 μ l \times 2 |
| (3) Antibody diluent | 20mL \times 1 |
| (4) Anti Diacetylspermine antibody concentrate(\times 100) | 60 μ l \times 1 |
| (5) HRP-anti Rabbit IgG Antibody concentrate(\times 80) | 80 μ l \times 1 |
| (6) OPD (o-phenyldiamine) tablets | 2 tab. |
| (7) Substrate solution | 30 mL \times 1 |
| (8) Stop solution | 15 mL \times 1 |
| (9) Wash buffer concentrate (\times 20) | 30 mL \times 1 |
| (10) Dilution plate | 1 plate |



[Equipments to be supplied by the user]

- (1) A microplate reader
- (2) A micropipet
- (3) A microplate washer

[Assay Method]

(1) Preparation of working solution

① Wash solution

Make sure that wash buffer concentrate does not contain any crystallized material prior to use. Working solution is prepared by dilution 30 mL of wash buffer concentrate with 570 mL of distilled deionized water. For convenience this solution can be kept at 2-8°C up to 14 days.

② Diacetylspermine Standard

Prepare 6 standards by serial dilution of diacetylspermine standard concentrate (200 nM) as followings

We recommend a polypropylene tube for preparation of standard 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)
Standard solution	200nM (μ L)	250	100	100	100	100	100
Deionized water	(μ L)	0	100	100	100	100	100

- ③ Anti Diacetylspermine antibody ($\times 100$)
Dilute $40 \mu\text{l}$ of Anti Diacetylspermine antibody concentrate ($\times 100$) with 4 mL of Dilution solution for 96 well reaction. Diluted antibody should not be stored.
- ④ HRP- anti Rabbit IgG Antibody ($\times 80$)
Dilute $65 \mu\text{l}$ HRP- anti Rabbit IgG Antibody concentrate ($\times 80$) with 5.2 mL of Dilution solution for 96 well reaction. Diluted antibody should not be stored..
- ⑤ 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.

(2) Preparation of urine sample

- ① Collect urine in sampling tube on demand. Add 0.1% Na_2N_3 at final concentration.
- ② After centrifugation at 1500rpm for 5min, dilute the resulted supernatant over 4 times with distilled deionized water.
- ③ Measure the amount of creatinine in remaining diluted supernatant for compensation.
※Prepared urine sample should be kept below -30°C if necessary .

(3) Assay procedure

- ① Pre-reaction
Prepare standard control wells containing $70\mu\text{L}$ of anti Diacetylspermine antibody solution and $70\mu\text{L}$ of 6 standards (200,100,50.0,25.0,12.5,6.25nM) in dilution plate. Likewise prepare experimental wells containing $70\mu\text{L}$ of anti Diacetylspermine antibody solution and $70\mu\text{L}$ 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 single measurement, reduce to $40\mu\text{L}$ of each solution.
- ② Preparation of reaction plate
 - ②-1 Add wash solution $300 \mu\text{l}$ to each well and wait another 30 minutes.
 - ②-2 Discard the wash solution from the wells completely and wash with $300 \mu\text{l}$ wash solution.
Repeat this step another 2 times
- ③ Primary reaction
 - ③-1 Apply $50 \mu\text{l/well} \times 2$ (In the case of measuring double wells) pre-reaction solution(See ①) and incubate for 1 hour.
 - ③-2 After the incubation, discard the reaction solution and wash with $300 \mu\text{l}$ wash solution. Repeat this step another 2 times.
- ④ Secondary reaction
 - ④-1 Apply $50 \mu\text{l}$ HRP - anti Rabbit IgG Antibody and incubate for 1 hour.
Equilibrate substrate buffer to room temperature prior to use.
 - ④-2 After incubation, discard the reaction solution and wash with $300 \mu\text{l}$ wash solution. Repeat this step another 2 times.
- ⑤ Coloring
Apply $100 \mu\text{l}$ Coloring solution to each well and incubate for 10 minutes at room temperature.

- ⑥ Stop reaction
Apply 100 μ l of Stop solution to stop the enzymatic reaction
- ⑦ Read absorbance
Read absorbance of 490nm or 492nm with a microplate reader .
- ⑧ Measure concentration
Measure the Diacetylspermine concentration using standard curve.

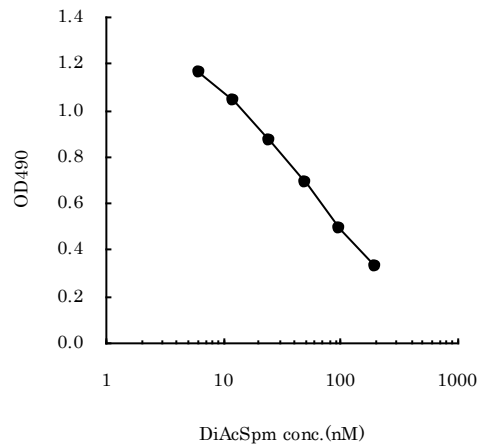
- * If actual measurements of sample exceed over 200nM, dilute those urine samples again as possible as to evaluate within the range of 6.25~200nM.
- * Concentration of diacetylspermine needs to be calculated from actual measurements by consideration of dilution ratio.
- * For the comparison of clinical data, actual measurements need compensation with the concentration of urinary creatinine (nmol/g · cre) .

Calculation method of clinical data

In general , the amount of urine change easily by amount of water take or environment even in renal disease patients and healthy persons, and concentrations of excrement depend on their amount. The amount of urinary creatinine depends on the amount of muscle and their measurements correlate positively. Therefore, correct actual measurement of Diacetylspermine concentration (nM) in urine with creatinine concentration (mg/dl) , as followings,

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

[Standard curve]



[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 precedures : $Y = 1.01X + 73.2$ $R^2=0.978$

[Usage notes]

- ① The Reagents should be stored at recommended temperature, -30°C .
- ② Do not use the reagents which is expired the date of usage.
- ③ Urine sample should be diluted more than 4 times with Dilution solution.
- ④ Do not leave the standard and Antibody for long time under room temperature.
- ⑤ The glassware for making coloring solution should be clean.
- ⑥ Since OPD (o-phenyldiamine) is harmful, handle with care.
- ⑦ Since Stop solution, 1N H_2SO_4 , is strong acid, handle with care.
- ⑧ The kit is constructed with well-adjusted combination in each lot. Replaced combination among different lots may cause unexpected results.
- ⑨ This kit is only for research use. Do not use for medicinal or any other purposees.
- ⑩ When using the reagents, take care to avoid them from touching to skin, mucous membrane, clothes, and getting into eye.
- ⑪ If the reagents happen to get into eye or mouth, wash out them and consult a doctor if you need.
- ⑫ After using the kit, wash your hand very carefully.
- ⑬ If you find that the packages of the reagents are broken or something wrong, do not use them.
- ⑭ When you store the reagents, make sure to avoid them from vaporizing, falling down.
- ⑮ After using the reagents, the packages should be discarded under the established rule.
- ⑯ We do not guarantee the quality of the packages and accompaniments if not used according this direction.

[Storage]

All reagents: -30°C

[References]

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- 2) Hiramatsu K, Sugimoto M, Kamei S, Hoshino M, Kinoshita K, Iwasaki K, and Kawakita M: Determination of amounts of polyamines excreted in urine; demonstration of N1,N8-diacetylspermidine and N1,N12-diacetylspermine as components commonly occurring in normal human urine.
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- 5) Hiramatsu K, Miura H, Kamei S, Iwasaki K, and Kawakita M: Development of a sensitive and accurate enzyme-linked immunosorbent assay(ELISA) system that can replace HPLC analysis for the determination of N1,N12- diacetylspermine in human urine.
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