

Urinary Diacetylspermidine ELISA Kit

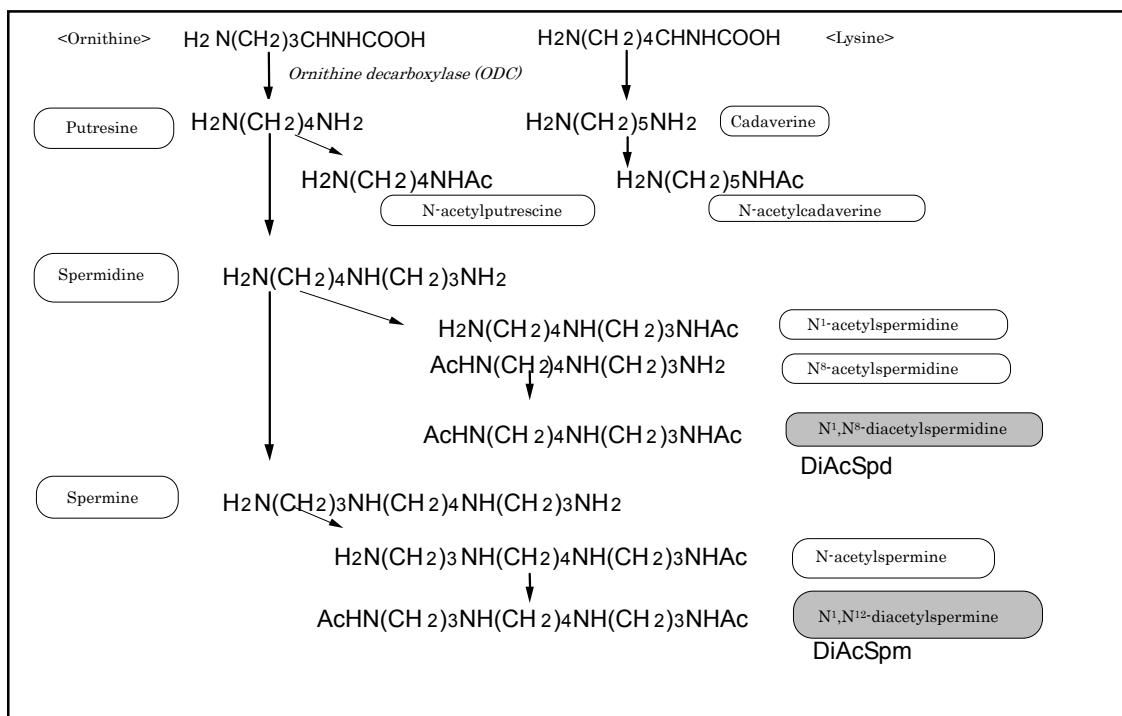
Polyamines are generally believed to function both in protein synthesis and DNA synthesis leading to control cell proliferation. In 1971, Russel firstly reported that total amount of urinary polyamines elevated in cancer patients. And quantitative kit of urinary polyamines were already developed and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

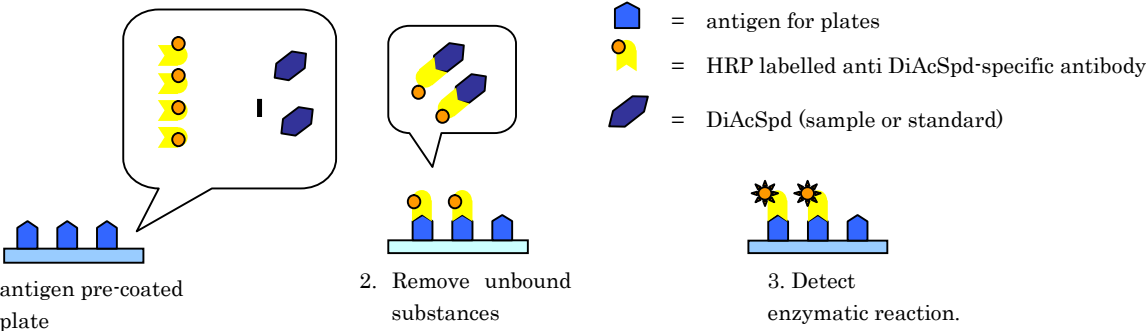
Our kit is convenient to quantify amount of urinary diacetylspermidine by using ELISA method. This kit is only for research use, not for diagnosis.

- Highly sensitive and specific
- Strip type well, antigen pre-coated microplate
- Assay range: 9.375~600nM



[measurement principle]

1. Incubate with sample.



[Kit Contents]

- | | |
|---|------------------------|
| (1) Antigen coated microtiter plate,96 wells | 1 plate |
| (2) Diacetylspermidine standard | 250 μ l \times 2 |
| (3) Reference standard and sample diluent | 15 mL \times 1 |
| (4) Antibody diluent | 20mL \times 1 |
| (5) HRP- anti Diacetylspermidine antibody concentrate(\times 100) 60 μ l \times 1
(mouse monoclonal antibody) | |
| (6) OPD (o-phenylendiamine) 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 Diacetylspermidine Standard

Prepare 6 standards by serial dilution of diacetylspermidine standard concentrate (600 nM) as followings

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

		300	150	75.0	37.5	18.75	9.375 (nM)
Standard solution	600nM (μ L)	100	100	100	100	100	100
Deionized water	(μ L)	100	100	100	100	100	100

- ③ HRP-anti Diacetylspermidine antibody ($\times 100$)
Dilute 40 μ l of Anti Diacetylspermidine antibody concentrate ($\times 100$) with 4 mL of Dilution solution for 96 well reaction. Diluted antibody should not be stored.
- ④ 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 Sample diluent.
- 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 μ L of anti Diacetylspermidine antibody solution and 70 μ L of 6 standards (600,300,150,75.0,37.5,18.75,9.375 nM) in dilution plate. Likewise prepare experimental wells containing 70 μ L of anti Diacetylspermidine antibody solution and 70 μ L of prepared urinary sample in the same plate. After settlement, incubate at room temperature for 30 minutes.
*Above reaction volumes can be applied for double measurements of primary reaction. If single measurement, reduce to 40 μ L of each solution.
- ② Preparation of reaction plate
- ②-1 Add wash solution 300 μ l to each well and wait another 30 minutes.
- ②-2 Discard the wash solution from the wells completely and wash with 300 μ l wash solution.
Repeat this step another 2 times
- ③ Primary reaction
- ③-1 Apply 50 μ 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 μ l wash solution. Repeat this step another 2 times.
- ④ Coloring
Apply 100 μ l Coloring solution to each well and incubate for 10 minutes at room temperature.
- ⑤ Stop reaction
Apply 100 μ l of Stop solution to stop the enzymatic reaction
- ⑥ Read absorbance
Read absorbance of 490nm or 492 nm with a microplate reader.
- ⑦ Measure concentration
Measure the Diacetylspermidine concentration using standard curve.
- * If actual measurements of sample exceed over 600nM, dilute those urine samples again as possible as to evaluate within the range of 18.75~600nM.

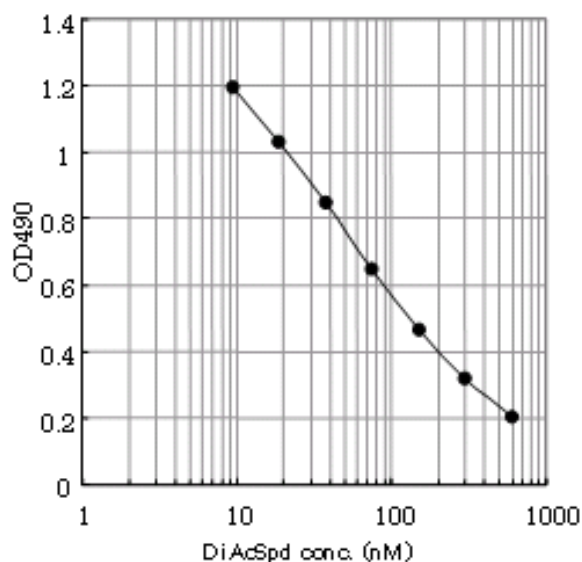
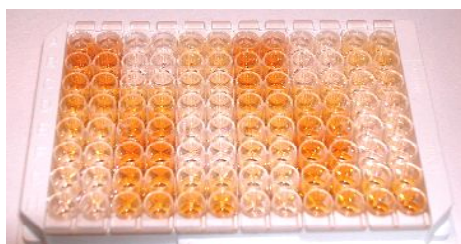
- * Concentration of diacetylspermine needs to be calculated from actual measurements by consideration of dilution ratio.
- * For the comparison of clinical data, actual measurements need compensation with the concentration of urinary creatinine (nmol/g · cre) .

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 Diacetylspermidine concentration (nM) in urine with creatinine concentration (mg/dl) , as followings,

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

[Standard curve]



[Reproducibility]

Domain of standard curve : 9.375~600nM

Minimum measurement range for detection: 18.75nM

Minimum dilution number of urine sample : ×2

Minimum sensitivity for detection: 37.5nM

Within-run (n=20, 2 concentration): CV (%) = 5.76, 2.58

Between-run (n=20, 2 concentration): CV (%) = 6.76, 4.25

Recovery test: In the recovery study, recoveries between 96% and 108% were obtained for 2, 4, 8times dilutions of the sample urine

Coexistence substance : No influence to Hemoglobin 4500mg/dL · Bilirubin 180mg/dL ·

Glucose 1000mg/dL · Ascorbic acid 40mg/dL

[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-phenylendiamine) is harmful, handle with care.
- ⑦ Since Stop solution, 1N H₂SO₄ 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 purposes.
- 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]

- 1) Russell DH:Increased polyamine concentration in the urine of human cancer patients.
Nature New Biol 233:144-145,1971
- 2) Hiramatsu K, Sugimoto M, Kamei S, Hoshino M, Kinoshita K, Iwasaki K, and Kawakita M:Determination of amounts of polyamines excreted in urine;demomstration of N1,N8-diacetylspermidine and N1,N12-diacetylspermine as components commonly occurring in normal human urine.
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This product is generated from GANP® mice.



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