SCETI

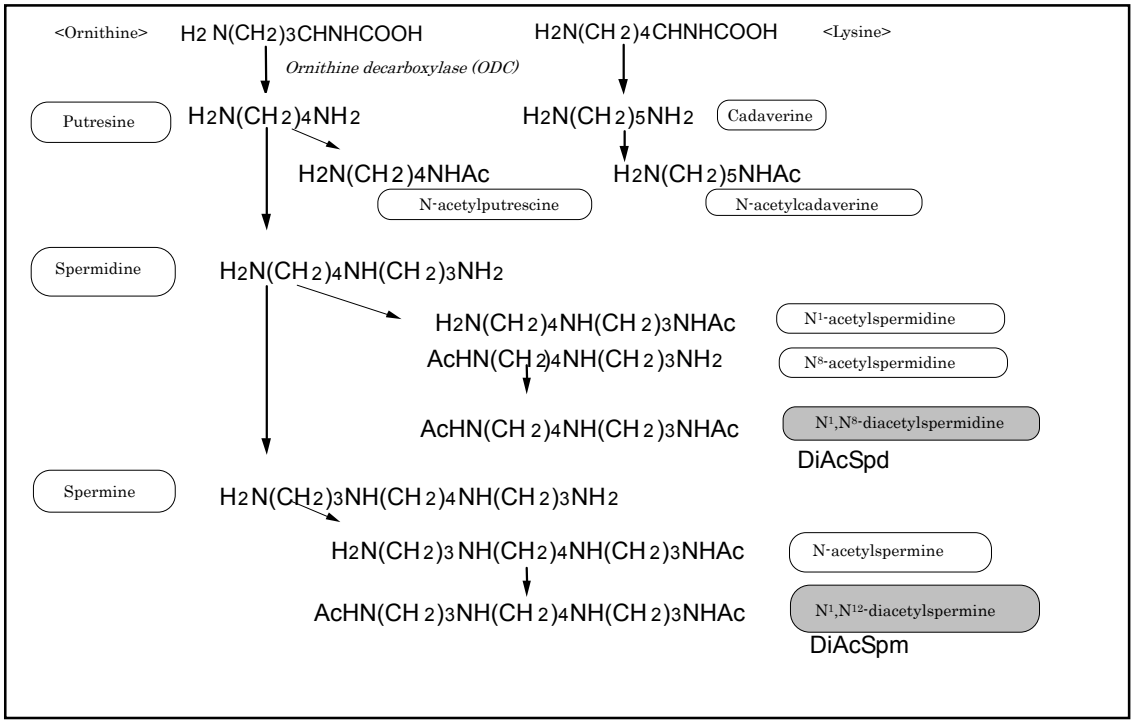
Code No.KK073

For research use only

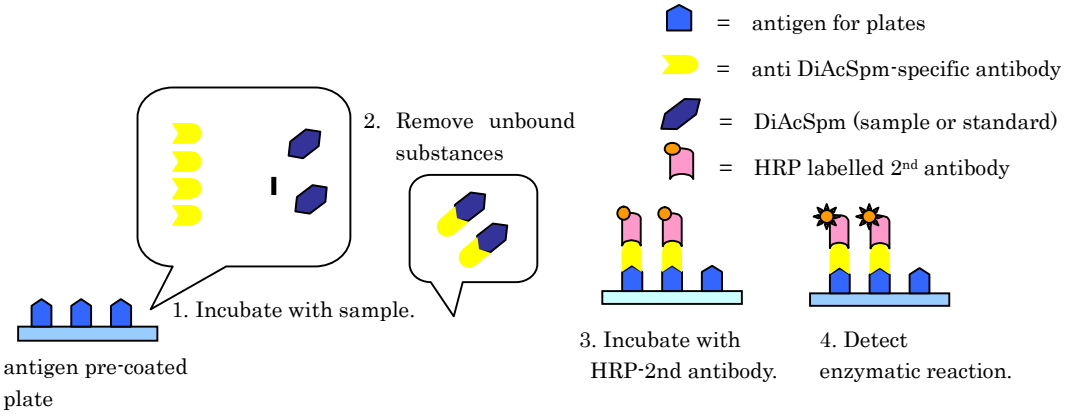
Urinary Diacetylspermine ELISA Kit

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

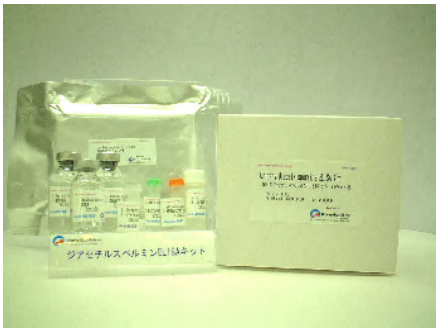


〔**measurement principle**〕



〔**Kit Contents**〕

(1)	Antigen coated microtiter plate,96 wells	1 plate
(2)	Diacetylspermine standard	250 μl × 2
(3)	Antibody diluent	20mL × 1
(4)	Anti Diacetylspermine antibody concentrate(×100)	60 μl × 1
(5)	HRP-anti Rabbit IgG Antibody concentrate(×80)	80 μl × 1
(6)	OPD (o-phenylendiamine) tablets	2 tab.
(7)	Substrate solution	30 mL × 1
(8)	Stop solution	15 mL × 1
(9)	Wash buffer concentrate (×20)	30 mL × 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℃ up t o 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	(μL)	250	100	100	100	100	100
Deionized water	(μL)	0	100	100	100	100	100

③ Anti Diacetylspermine antibody (×100)
Dilute 40μl of Anti Diacetylspermine antibody concentrate (×100) with 4 mL of Dilution solution for 96 well reaction. Diluted antibody should not be stored.

④ HRP- anti Rabbit IgG Antibody (×80)
Dilute 65μl HRP- anti Rabbit IgG Antibody concentrate (×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 centrifuge 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℃ if necessary .

(3) Assay procedure

① Pre-reaction

Prepare standard control wells containing 70μL of anti Diacetylspermine antibody solution and 70μL of 6 standards (200,100,50.0,25.0,12.5,6.25nM) in dilution plate. Likewise prepare experimental wells containing 70μL of anti Diacetylspermine antibody solution and 70μL 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 40μL of each solution.

② Preparation of reaction plate

②-1 Add wash solution 300μl to each well and wait another 30 minutes.

②-2 Discard the wash solution from the wells completely and wash with 300μl wash solution.

Repeat this step another 2 times

③ Primary reaction

③-1 Apply 50μl/well×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 300μl wash solution. Repeat this step another 2 times.

④ Secondary reaction

④-1 Apply 50μl 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 300μl wash solution. Repeat this step another 2 times.

⑤ Coloring

Apply 100μl 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.

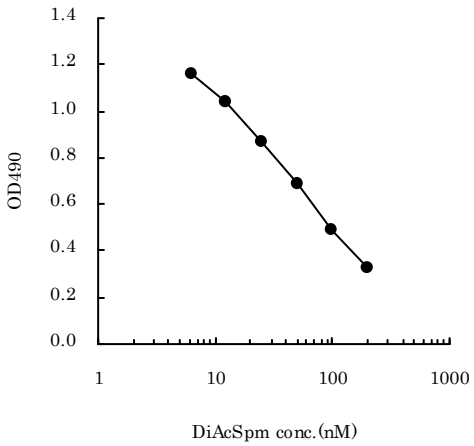
* **If actual measurements of sample exceed over200nM, dilute those urine samples again as possible as to evaluate within the range of 6.25～200nM.**
* **Concentration of diacetylspermine needs to be calculated from actual measurements by consideration of dilution ratio.**
* **For the comparison of clinical 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 donor, 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,

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

〔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 values Y = 1.01X + 73.2　R^2=0.978

〔**Usage notes**〕

① The Reagents should be stored at recommended temperature, －30℃.
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 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 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℃

〔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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J. Cancer Res. Clin. Oncol., 123:539-545,1997

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 HLPC analysis for the determination of N1,N12- diacetylspermine in human urine.
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Supplier

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