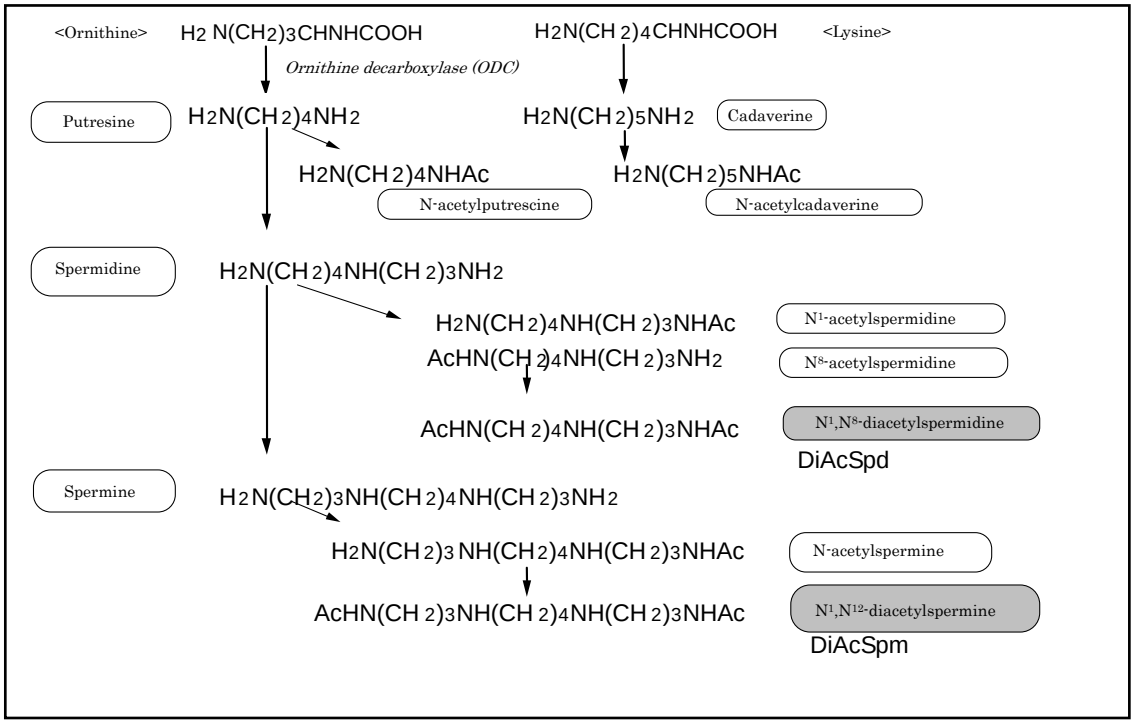
Trans Genic Inc.
Code No.KK073

For research use only

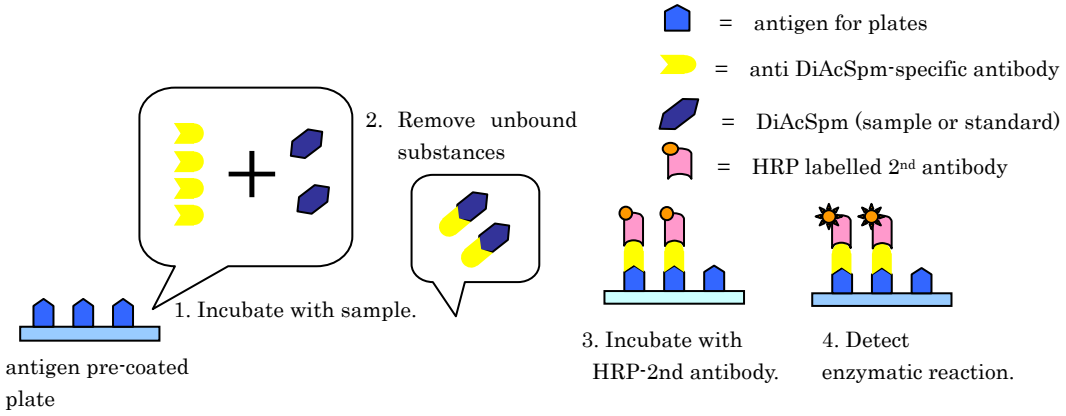
Urinary Diacetylspermine ELISA Kit

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 kits 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: 6.25～200 nM

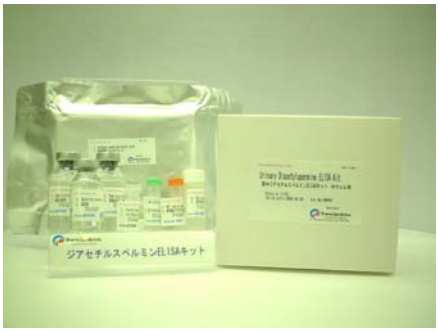


〔**measurement principle**〕



〔**Kit Contents**〕

(1)	Antigen coated microtiter plate, 96 wells	1 plate	
(2)	Diacetylspermine standard	250 µL	× 2
(3)	Antibody diluent	20 mL	× 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 tablets	
(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) Micropipets
(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 follows

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 200 nM	(µL)	250	100	100	100	100	100	
Deionized water	(µL)	0	100	100	100	100	100	

③ Anti Diacetylspermine antibody (×100)
Dilute 40 µL 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 65 µL 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 solution 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% NaN_3 at final concentration.

② After centrifugation at 1500 rpm for 5 min, 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 µL of anti Diacetylspermine antibody solution and 70 µL of 6 standards (200, 100, 50.0, 25.0, 12.5, 6.25 nM) in dilution plate. Likewise prepare 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 single measurement, reduce to 40 µL of each solution.

② Preparation of reaction plate
②-1 Add wash solution 300 µL to each well and wait another 30 minutes.
②-2 Discard the wash solution from the wells completely and wash with 300 µL wash solution.
Repeat this step another 2 times

③ Primary reaction
③-1 Apply 50 µL/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 300 µL wash solution. Repeat this step another 2 times.

④ Secondary reaction
④-1 Apply 50 µL 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 µL wash solution. Repeat this step another 2 times.

⑤ Coloring
Apply 100 µL 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 absorbance
Read absorbance of 490 nm or 492 nm with a microplate reader.

⑧ Measure concentration
Measure the Diacetylspermine concentration using standard curve.

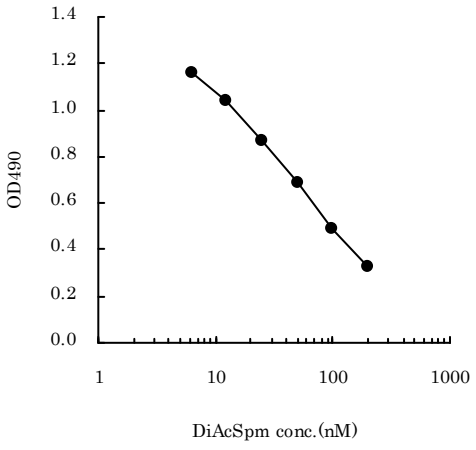
* **If actual measurements of sample exceed over 200 nM, dilute those urine samples again as possible as to evaluate within the range of 6.25～200 nM.**
* **Concentration of diacetylspermine needs to be calculated from actual measurements by consideration of dilution ratio.**
* **For the comparison of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

Calculation method of clinical data

The concentrations of excrement in urine depend on the urine volume which changes by the amount of water take or environment in both renal disease patients and healthy persons. In general, the measurements of urinary excrement need to be corrected by the urinary creatinine because the amount depends on that of muscle and the measurement is able to convert into the amount per 1g creatinine. Therefore, correct actual measurement of Diacetylspermine concentration (nM) in urine with creatinine concentration (mg/dL), as follows

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

〔Standard curve〕



〔**Reproducibility**〕

Domain of standard curve : 6.25～200 nM
Minimum measurement range for detection : 12.5 nM
Minimun dilution number of urine sample : ×4
Minimum sensitivity for detection : 50.0 nM
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, 8 times dilutions of the sample urine
Coexistent substance : No influence to Hemoglobin 400 mg/dL・Bilirubin 10 mg/dL・Glucose 1000 mg/dL・Ascorbic acid 100 mg/dL
Comparison between the ELISA kit and HPLC procedures : Y = 1.01 X + 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 antibodies 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 1 M 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 eyes.
⑪ If the reagents happen to get into eyes or mouth, wash out them and consult a doctor if you need.
⑫ After using the kit, wash your hands 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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3) Sugimoto M, Hiramatsu K, Kamei S, Kinoshita K, Hoshino M, Iwasaki K, and Kawakita M: Significance of urinary N1,N8-diacetylspermidine and N1,N12- diacetylspermine as indicators of neoplastic diseases.
J. Cancer Res. Clin. Oncol.,121:317-319,1995

4) Hiramatsu K, Sugimoto M, Kamei S, Hoshino M, Kinoshita K, Iwasaki K, and Kawakita M: Diagnostic and prognostic usefulness of N1,N8- diacetylspermidine and N1,N12- diacetylspermine in urine as novel markers of malignancy.
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.
J. Biochem., 124:231-236,1998

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