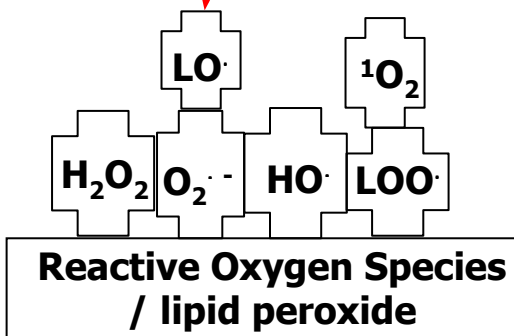


**8-OHdG Pretreatment Kit (US-001)
- Technical Explanation with Data -**

**Bio Healthcare Department
TANITA Corporation**

What's the oxidative stress ?

The body is damaged by oxidative stress every day!



SOD	Vitamin E	
GSH-Px	Carotenoid	Uric acid
Catalase	Flavonoid	Vitamin C
Other antioxidative enzymes	Other antioxidants	

Antioxidants

An imbalance means...

We intake antioxidants from food!

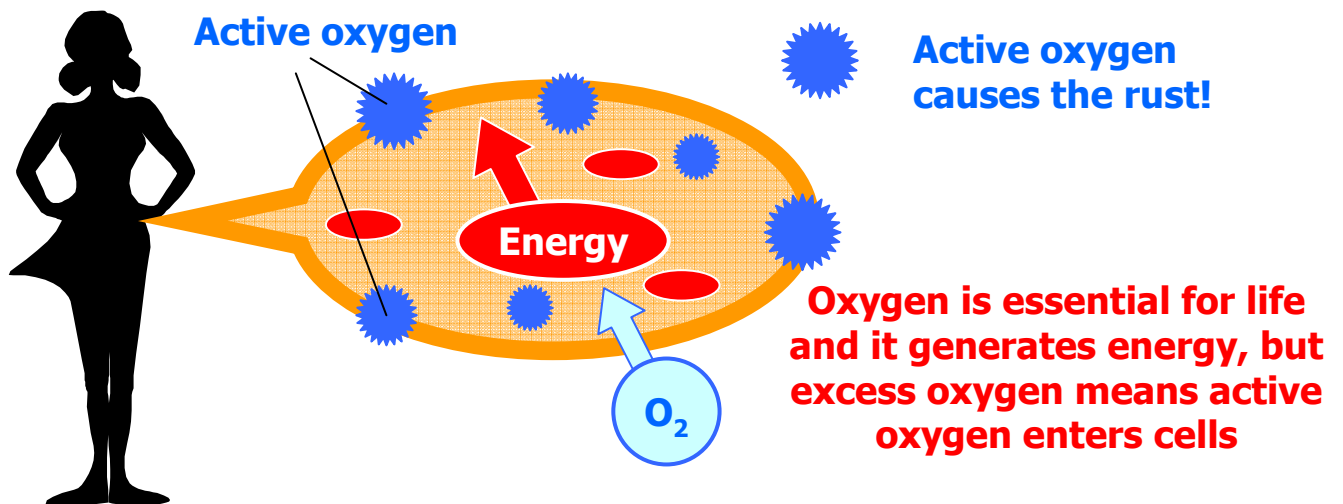
We go into a state of oxidative stress

→ **Oxidative stress is a factor in the pathogenesis of illness and a trigger of aging.**

Source: "GANYOBO-SYOKUHIN" Osawa et. al, (partial modification),
 "KASSEI-SANSO" Kondo et. al,

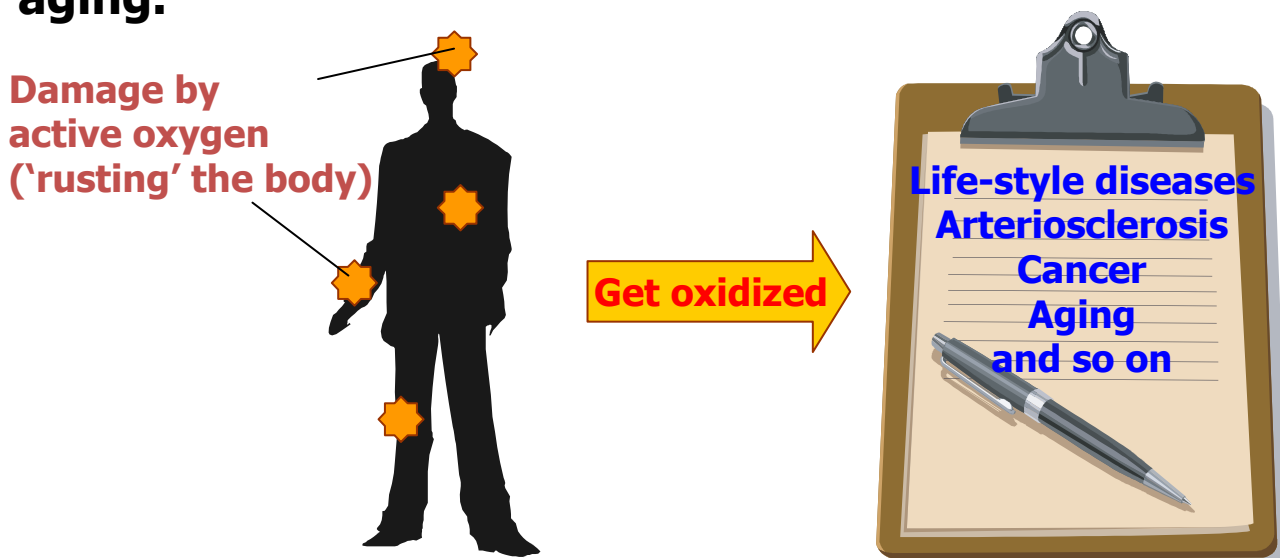
What's the oxidative stress ?

When reactive oxygen species such as lipid peroxides are taken into the body, they inflict damage (they 'rust' the body). Oxidative stress is an excessive state of rust in the body.



What does oxidative stress do to our bodies?

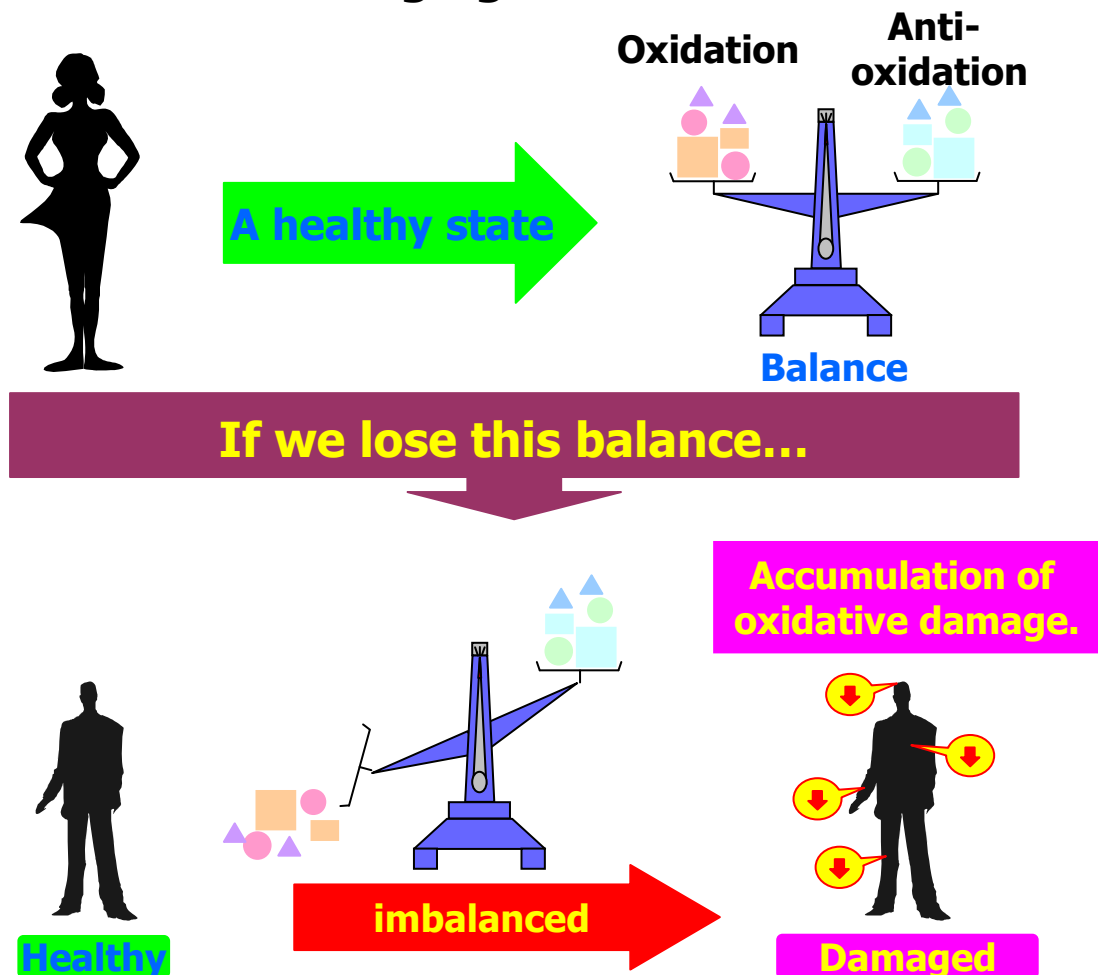
Oxidative stress increases 'damage (rust) in the body'. Excessive oxidative damage is considered to be involved in lifestyle diseases (cancer, diabetes, myocardial infarction, cerebral infarction, arteriosclerosis, etc.) and aging.



Is active oxygen really so bad?

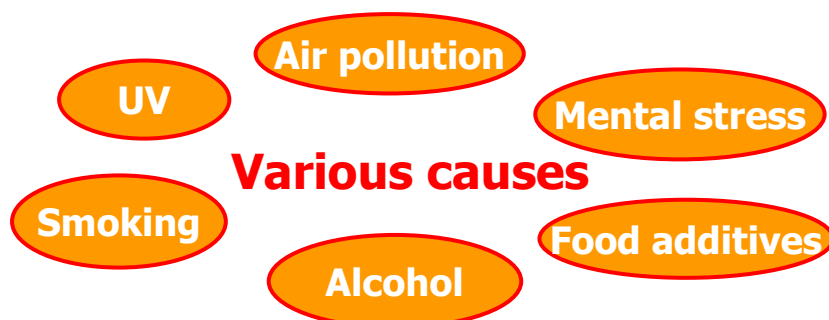
Active oxygen plays an important role in immune function such as fighting infections.

However, surplus active oxygen causes, for example, lifestyle diseases and aging.

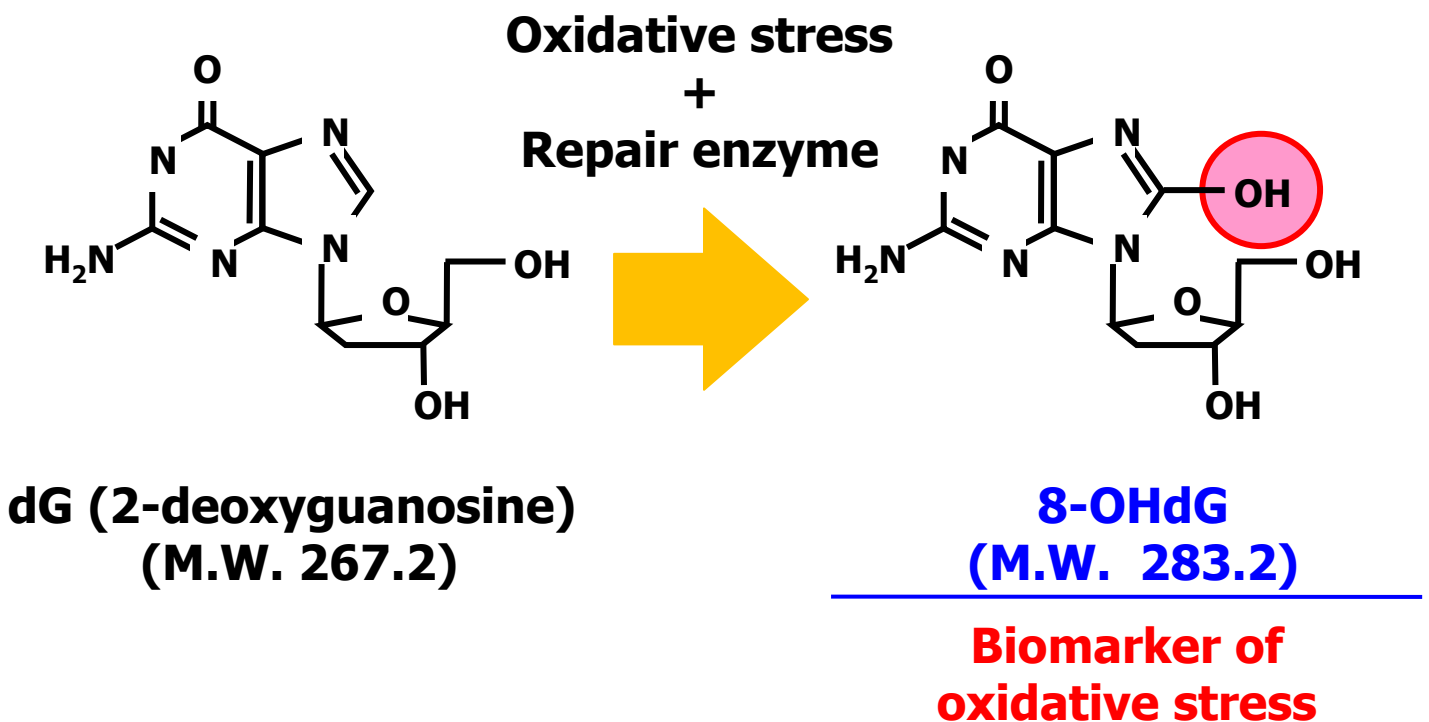


Causes to lose the balance:

Life-style habits, air pollution, mental stress and so on.



8-hydroxy-2'-deoxyguanosine (8-OHdG)



8-OHdG concentration in human biospecimens

Urine	2 - 50 ng/mL
Serum/Plasma	0.05 - 10 ng/mL

Increase in the amount of 8-OHdG → **Liable to diseases**

Intestinal cancers, Diabetes, Alzheimer's disease and so on.

Relationship between 8-OHdG and diseases

*** associated with many diseases (cancer, life-style diseases)**

Subjects	Urinary 8-OHdG vs. healthy person
Cancer	
Bladder cancer	2 times
Prostate cancer	1.6 times
Lung cancer	1.4 times
Leukaemia	2.1 times
Other diseases except for cancers	
Diabetes (HbA _{1c} > 5.8%)	2.4 times
Diabetes (Type 1)	1.3 times
Articular rheumatism	1.5 times
Atopic dermatitis	1.9 times
Lung disease	
Chronic obstructive lung disease	1.5 times
Neural/muscle disease	
Amyotrophic lateral sclerosis (ALS)	1.6 times
Ataxia (Friedreich type)	3 times
Hazardous substance exposure	
Arsenic (Chronic arsenic poisoning)	1.3 times
Chromium (+6)	1.6 times
Others	
Smoking	1.3 times
Drinking	1.5 times

Pre-treatment kit



Product name: 8-OHdG treatment kit

Code: US-001

JAN 4904765410336

ITF 410335

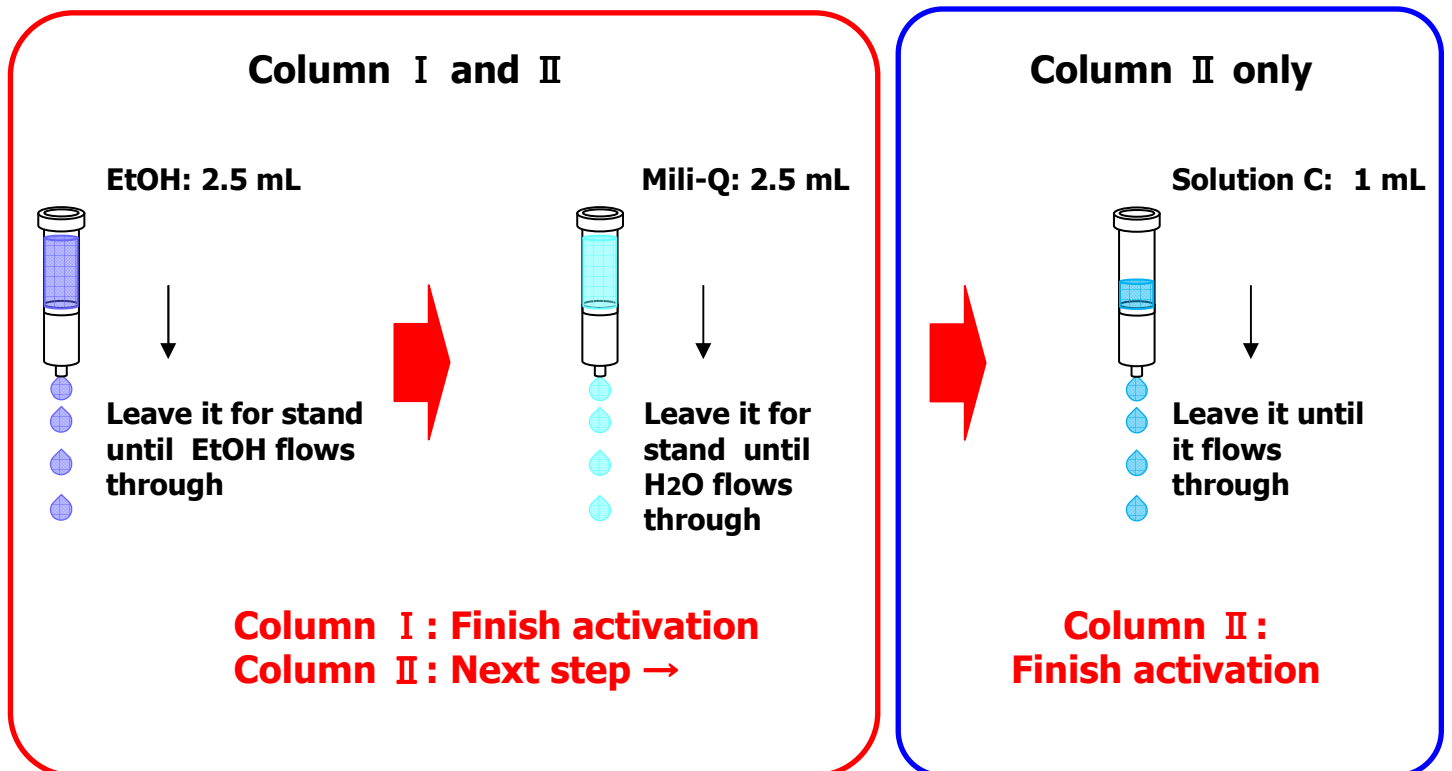
Packing size : for 20 samples

***An HPLC system, electrochemistry detector, analysis column are necessary for the measurement.**

Measurable samples

Samples	Urine	Serum/plasma
Range of concentration	1- 200 ng/mL	0.01- 10 ng/mL
Pre-treatment (Prior to US-001)	Centrifuge (4 °C, 8400 rpm, 10 min)	Centrifuge (R. T., 3000 rpm, 10 min)
Sample quantity (1 sample)	0.9 mL	1.1 mL

Activation of pre-treatment column

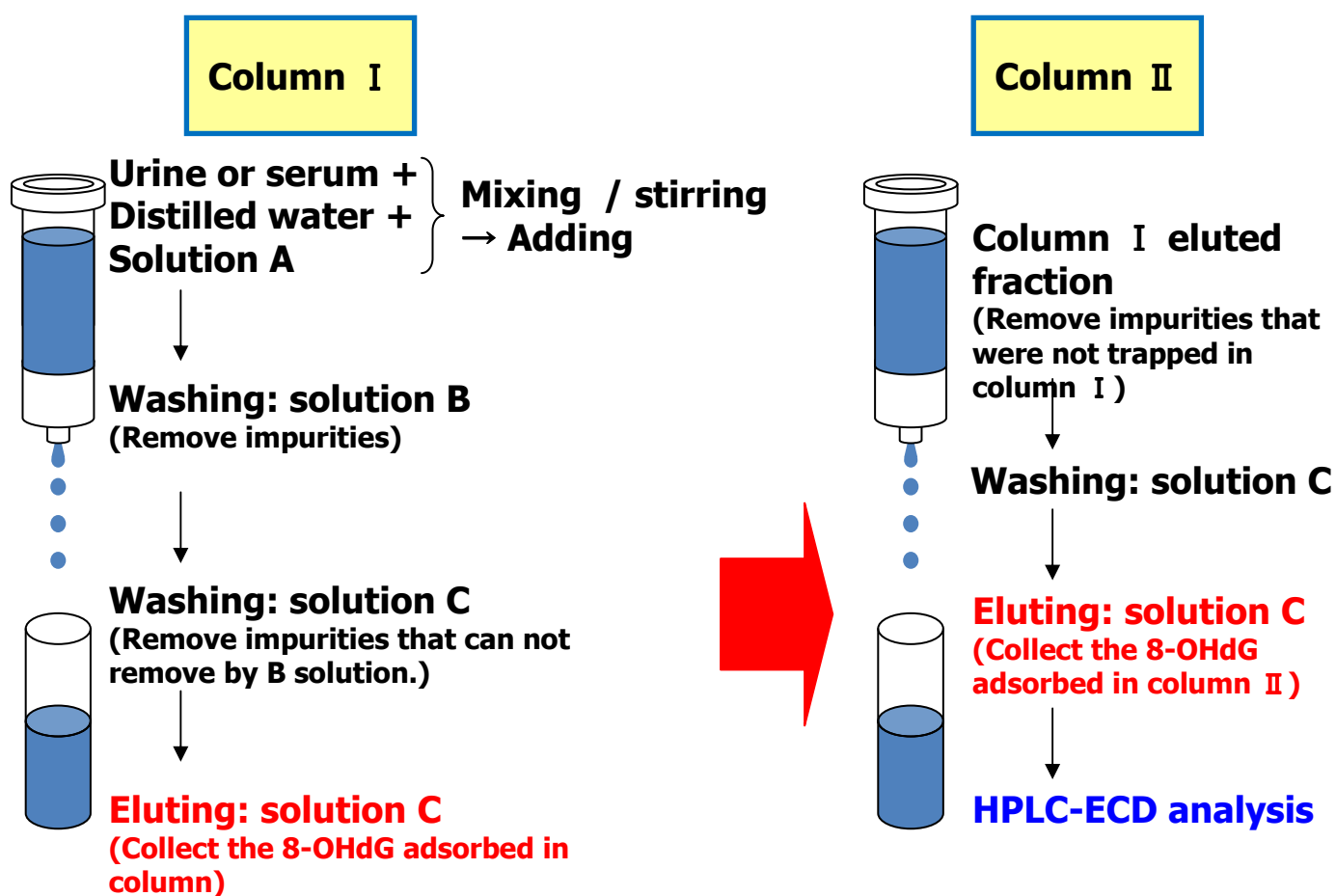


Caution!: After the activation, go forward to next work immediately! Do not make the column dry.



Pre-treatment method of samples by US-001

US-001 facilitates the measurement of 8-OHdG, because impurities in samples are removed by Column I and II.



**Typical pretreatment time: 2 hours 45min.
(Parallel treatment is available, which enables to treat multi samples simultaneously.)**

**Temperature of the pretreatment:
23- 27 °C (Recommended temperature: 23- 24 °C)**

Treatment of urine sample

Urine sample: 10 ml / person



Gathering



Centrifuge
4 °C, 8400 G, 10 min.



Precipitate
(to be discarded)



Supernatant
Storage at -20 °C

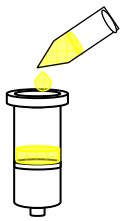


Supernatant

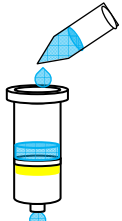
Pre-treatment method of urine sample using US-001

Column I

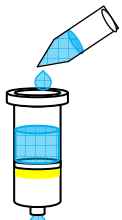
Urine: 0.9 ml + H₂O: 1.35 ml + Solution A: 0.75 ml
→ Mixing / Stirring (Sample X)



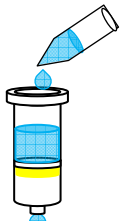
↓ Load 2 ml Sample X to activated column I



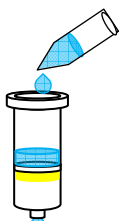
↓ Load 2 ml B solution (Leave for 15 min.)



↓ Add 2 ml B solution (Leave for 5 min.)



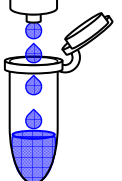
↓ Add 2 ml B solution



↓ Load 0.9 ml C solution



↓ Load 0.7 ml C solution

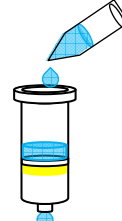


Collect eluted solution (Sample Y)

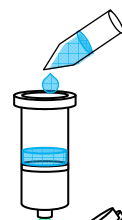
Column II



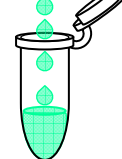
↓ Load 0.7 ml Sample Y to activated column II



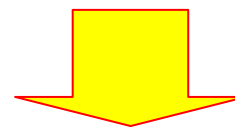
↓ Load 0.4 ml C solution (Leave for 10 min.)



↓ Load 0.8 ml C solution



Collect eluted solution (Sample Z)



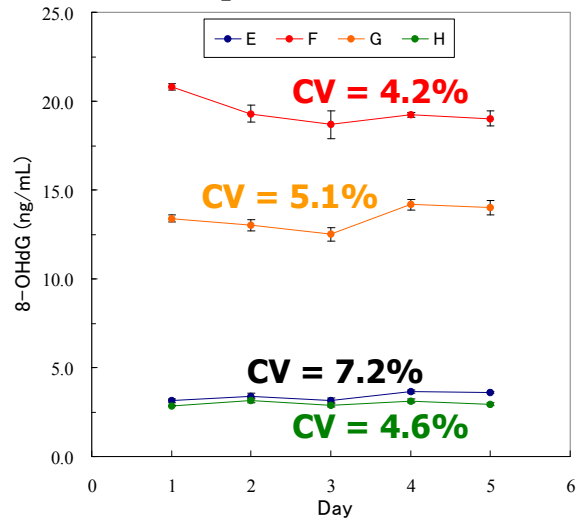
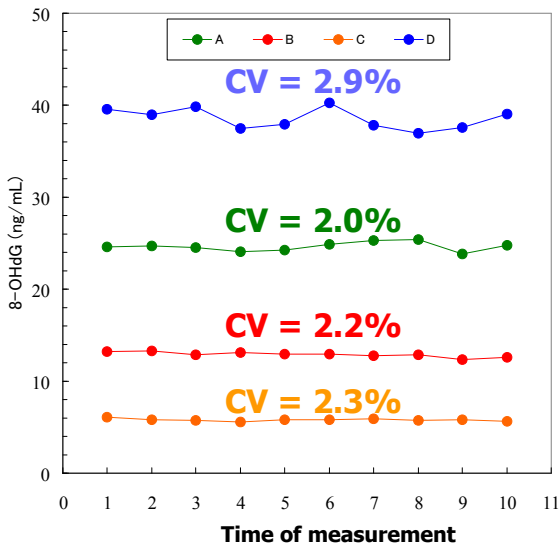
HPLC-ECD analysis
Injection volume: 15 μ l

Recommended pretreatment temperature: 23- 24 °C



HPLC-ECD system

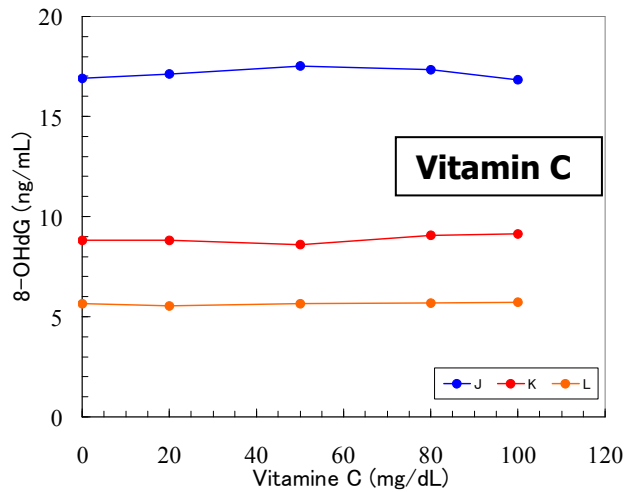
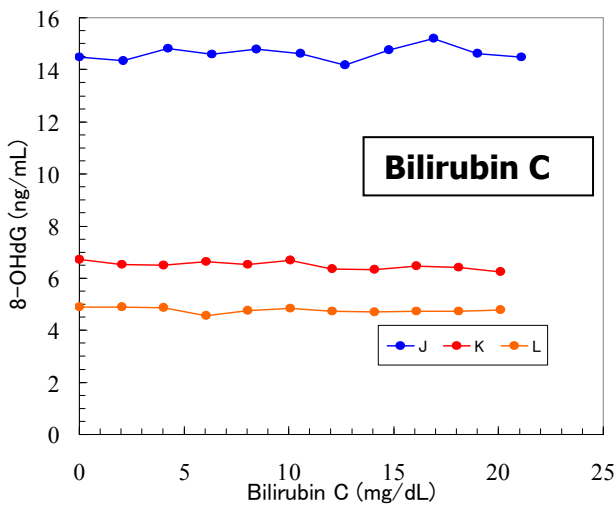
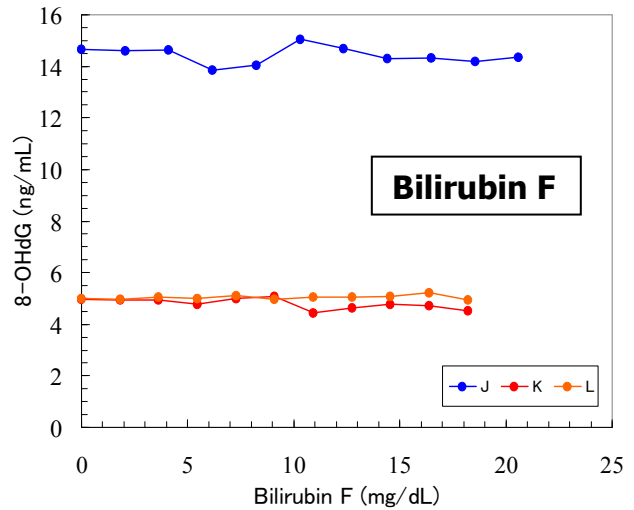
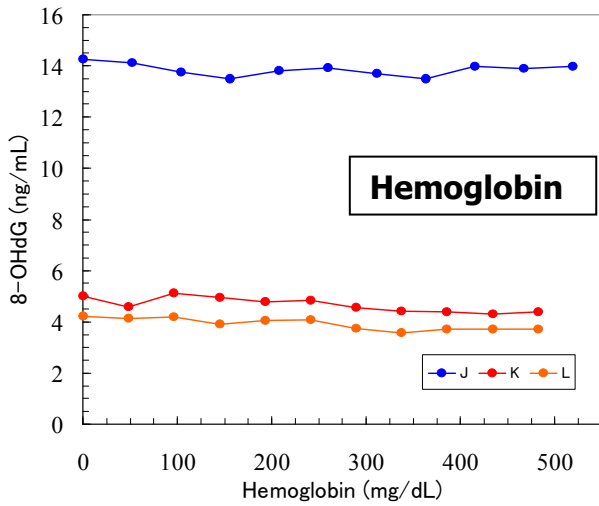
Basic data of urine sample 1



Reproducibility

Repeatability

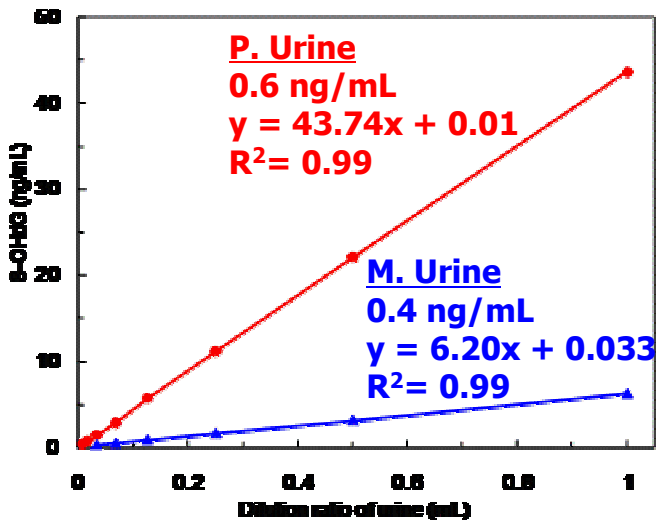
Good reproducibility and repeatability (CV values: less than 7 %)



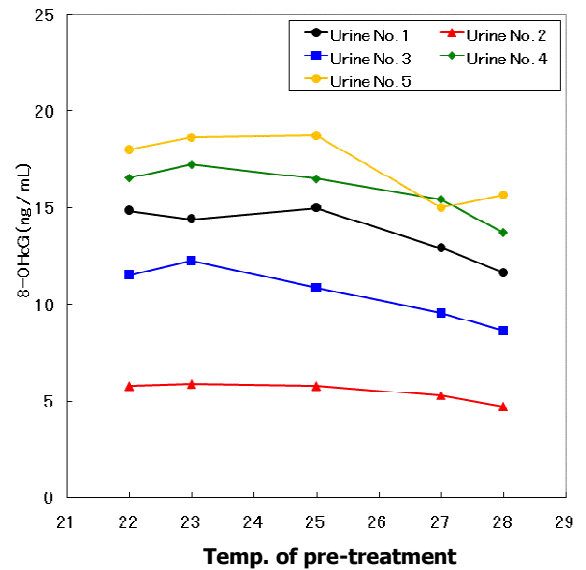
Influence of interfering substances

Quite small influence by interfering substances

Basic data of urine sample 2

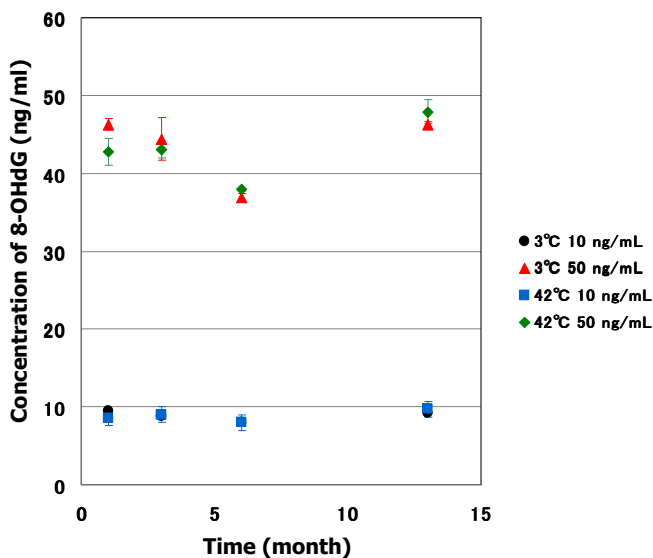


Dilution linearity

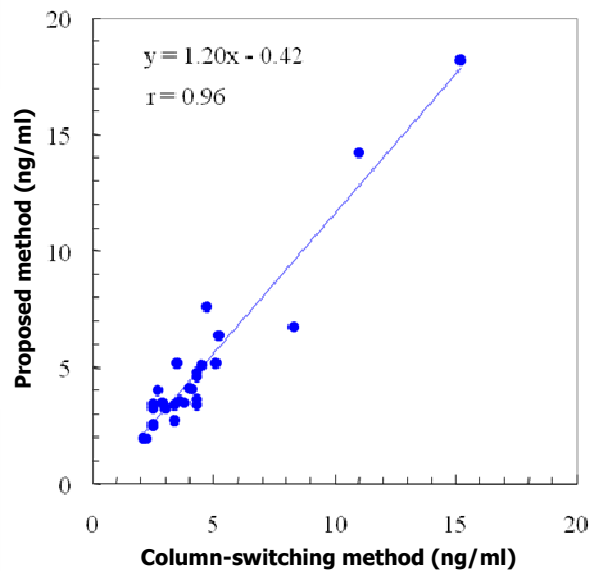


Effect of temp.

Low detection limit of urine sample: 0.4 ml, Dynamic range: 0.4 – 200 ng/ml
Temperature of pre-treatment operation: 22 – 27 °C



Long-term stability



Relationship between column-switching method and proposed method

Long-term stability: 12month at 3 – 42 °C
The correlation between column-switching method and proposed method is excellent.

**Treatment of blood sample
(Serum/plasma sample)**

Venous blood: 10 ml / person



**Blood sample tube
(with or without heparin)**



**Serum: Leave it for 30min. at room temp.
Plasma sample: Put in the icy water for a
few min.**



**Centrifuge:
2500- 3000 rpm, 5- 10 min.**

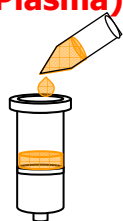


**Supernatant
Storage at -80 °C**

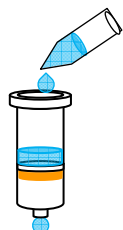
Pre-treatment method of serum (plasma) sample using US-001

Column I

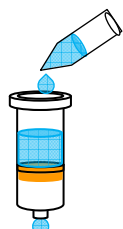
1.1 ml Serum + 0.55 ml H₂O + 0.55 ml Solution A
(or Plasma) → Mixing / Stirring (Sample X)



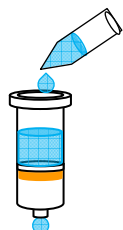
Load 2 ml Sample X
to activated column I



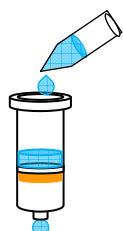
Load 2 ml B solution
(Leave for 15 min.)



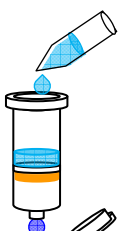
Add 2 ml B solution
(Leave for 15 min.)



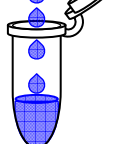
Add 2 ml B solution



Load 0.9 ml C solution

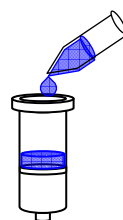


Load 0.7 ml C solution

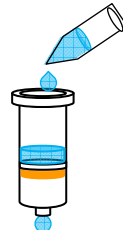


Collect eluted solution (Sample Y)

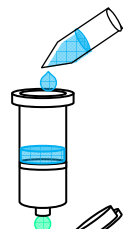
Column II



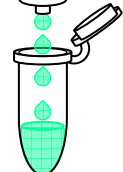
Load 0.7 ml Sample Y
to activated column II



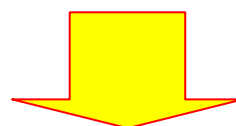
Load 0.4 ml C solution
(Leave for 10 min.)



Load 0.8 ml C solution



Collect to eluted solution (Sample Z)

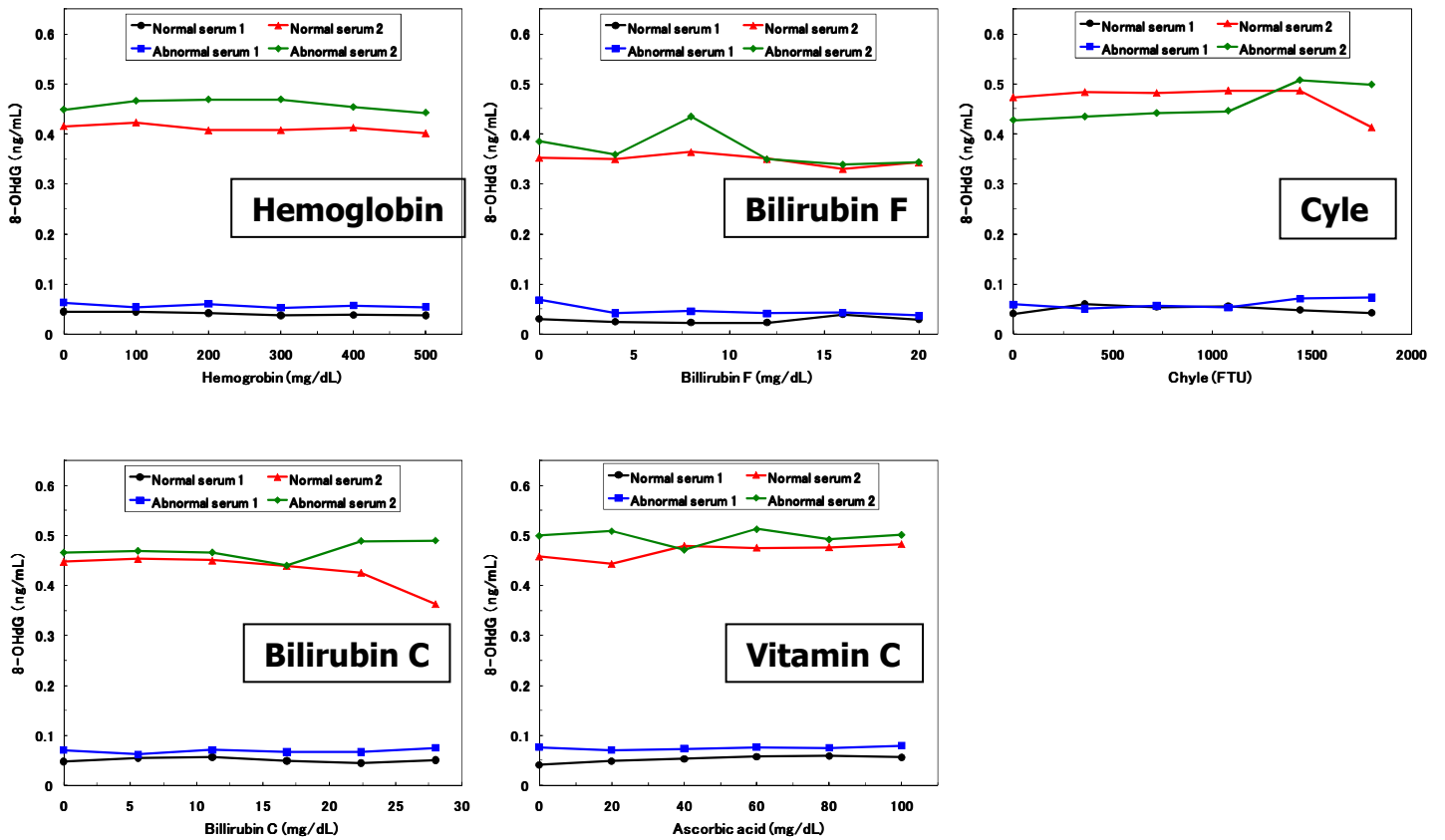


HPLC-ECD analysis
Injection volume: 100 μ l

Recommended pretreatment
temperature: 23- 24 °C

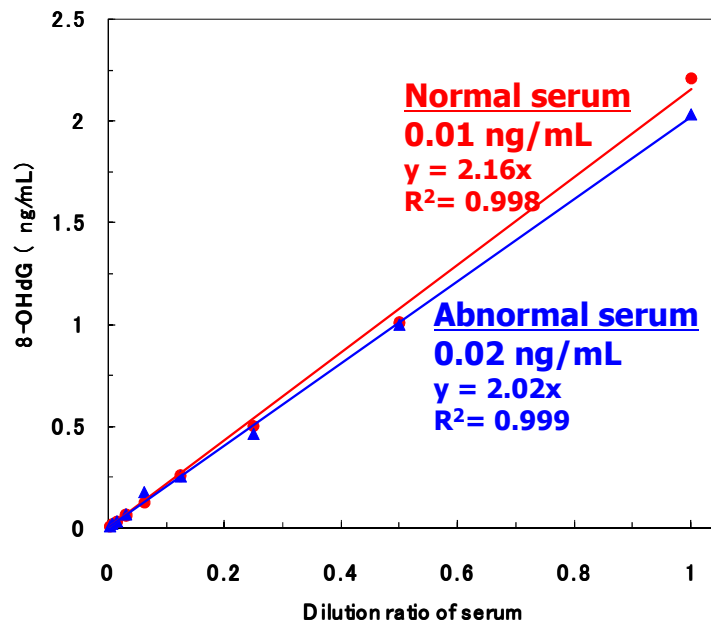
Notice: Some injected samples may give a large signal that prevents measurement. In such case, use the following procedure to repeat the analysis:
0.4 ml sample Z + 0.1 ml Solution A
→ Mixing → HPLC analysis

Basic data of serum sample 1



Influence of interfering substances

Little influence by interfering substances.



Dilution straightness

Low detection limit of serum samples: less than 0.01 ng/ml

Concentrations of 8-OHdG in serum for healthy people

	Age/sex	HPLC-ECD (pg/ml)	ELISA (pg/ml)		Age/sex	HPLC-ECD (pg/ml)	ELISA (pg/ml)
1	40/M	9.91	N. D. (90)	21	30/M	29.10	N. D. (10)
2	30/M	10.37	N. D. (80)	22	40/M	32.94	N. D. (20)
3	30/M	18.01	N. D. (90)	23	20/M	45.45	N. D. (20)
4	30/M	24.43	N. D. (100)	24	40/M	40.73	N. D. (20)
5	30/M	23.37	N. D. (80)	25	30/M	11.25	N. D. (10)
6	30/M	19.78	N. D. (80)	26	40/M	20.30	N. D. (10)
7	20/F	5.56	N. D. (70)	27	30/M	22.09	N. D. (10)
8	40/M	19.42	N. D. (80)	28	30/M	23.05	N. D. (10)
9	40/M	13.40	N. D. (100)	29	40/F	15.32	N. D. (10)
10	20/M	30.60	N. D. (110)	30	30/M	29.05	N. D. (10)
11	40/M	17.50	N. D. (80)	31	30/M	44.88	N. D. (10)
12	30/M	33.79	N. D. (80)	32	30/M	26.17	N. D. (10)
13	40/M	51.86	N. D. (90)	33	20/F	12.32	N. D. (10)
14	30/M	38.53	N. D. (90)	34	40/M	69.01	N. D. (10)
15	40/M	13.52	N. D. (100)	35	30/M	42.39	N. D. (50)
16	40/F	9.99	N. D. (80)	36	20/M	45.20	N. D. (20)
17	30/M	25.33	N. D. (90)	37	20/F	18.64	N. D. (10)
18	40/F	14.46	N. D. (120)				
19	40/F	16.53	N. D. (20)				
20	30/M	20.62	N. D. (10)				

M: male, F: female, N. D.: not detected, (): reference value

ELISA method is unable to detect 8-OHdG concentration in serum in healthy people, because the detection limit of 130 pg/ml was higher than the normal range for healthy people. The 8-OHdG concentration in serum in healthy people was in the range of 0 to 70 pg/ml (25.5 ± 13.8 pg/ml, n= 37).

Setting condition of recommended HPLC system

(1) Recommended HPLC system

1. HPLC system: LC-2010 series (SHIMAZU), LC-8020 series (TOSO) and so on.
2. Pump: LC-20AD (SHIMAZU), DP-8020 (TOSO)
3. Degasser: DGU-20A3 (SHIMAZU), SD-8022 (TOSO)
4. Auto injector: SIL-20A (SHIMAZU), AS-8020 (TOSO)
5. Column oven: CTO-20AC (SHIMAZU), CO-8020 (TOSO)
6. Electrochemical detector: NANOSPACE 3005 (SHISEIDO), ECD-700(EiCOM), ED-703 (GL science), Coulochem III (esa) and so on.
7. Analysis column: Develosil C30 (5 μ m, 4.6 \times 250mm) (NOMURA),
Guard column: Develosil C30 (3 μ m, 1.0 \times 4.0mm) (NOMURA)

*Serum sample: verified only LC-2010 series

(2) Recommended HPLC setting condition

1. Flow rate: 1.0 mL/min
2. Temp. of column oven: 27 °C
3. Applied potential: except for Coulochem III: +500 mV
(Working electrode (WE): Glassy carbon, Reference electrode (RE): Ag/AgCl)
Coulochem III: +300 mV
(Guard cell electrode: +300 mV, E₁ 0 mV, WE: Pd, RE: Pd)
4. Pump condition: 0 -15.5 min, mobile phase A: 100%
15.5- 16.5 min, mobile phase B: 100%
16.5- 40 min, mobile phase A: 100%
5. Mobile phase A:
35 mmol/l phosphate buffer (pH 7.0) containing 5 % acetonitrile and 30 μ mol/l EDTA
Mobile phase B:
35 mmol/l phosphate buffer (pH 7.0) containing 50% acetonitrile and 30 μ mol/l EDTA

* Serum sample: verified only coulochem III

Procedures of pre-treatment (photo)



Mixing of sample

Pre-treatment by column II

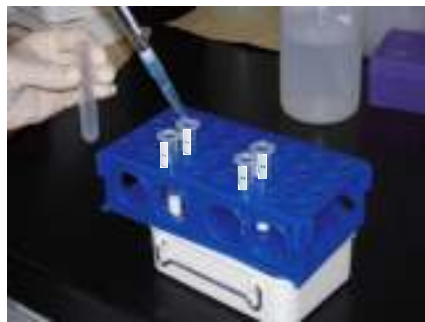


Loading eluted
sample to column
II

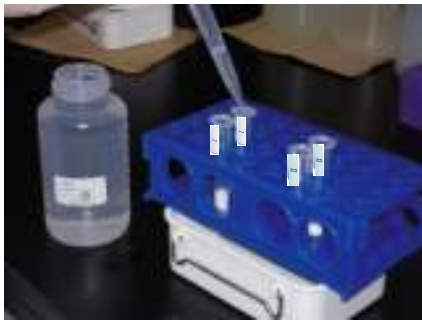


Eluting:
solution C

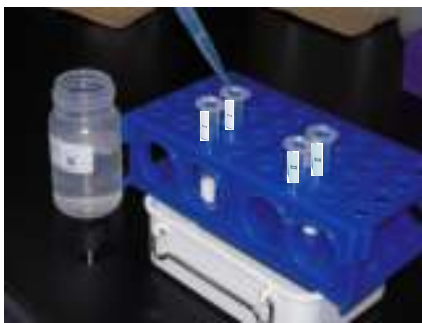
HPLC-ECD analysis



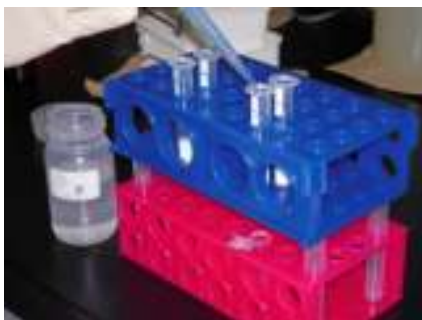
Loading sample
to column I



Washing:
solution B



Washing:
solution C

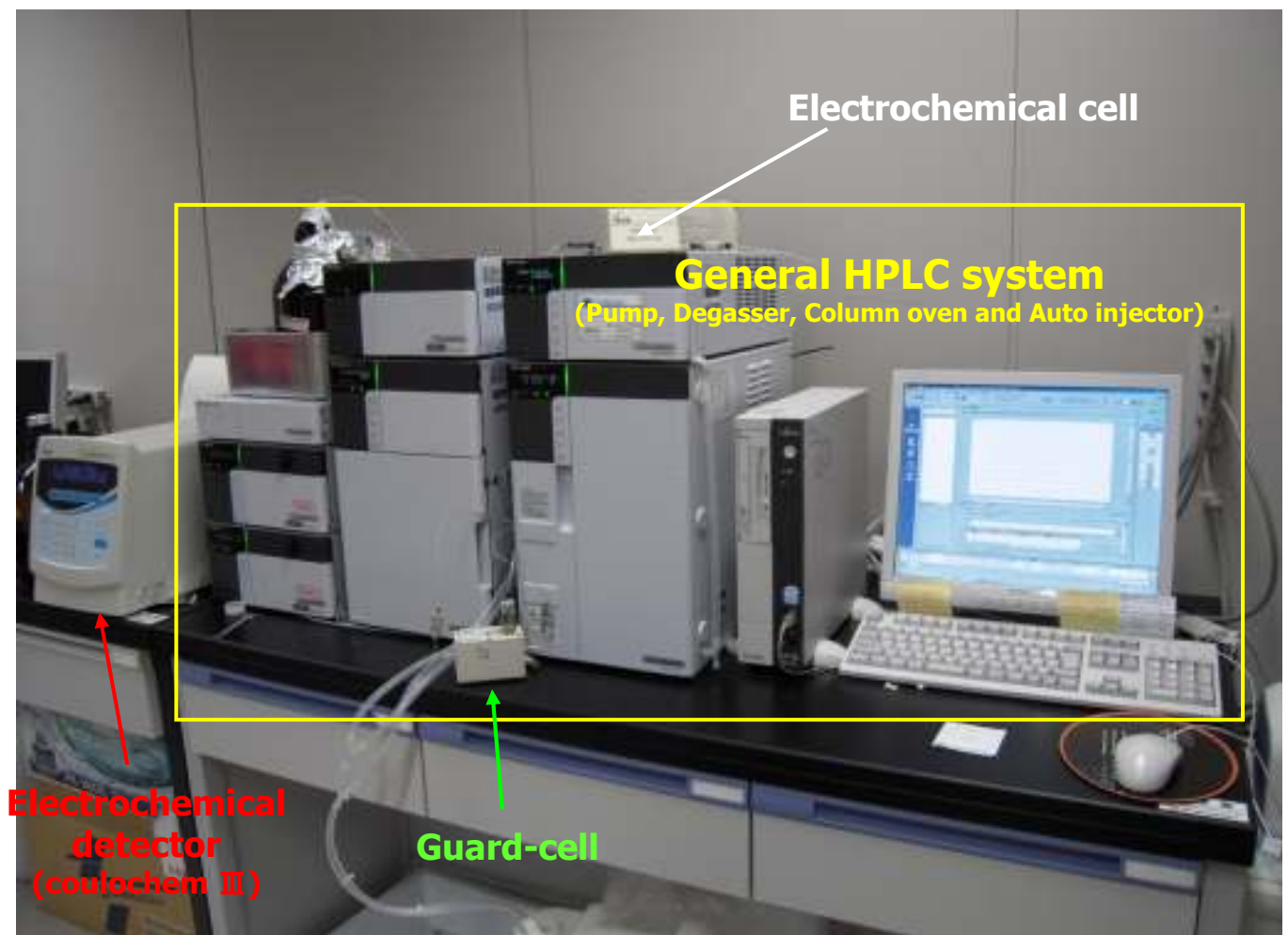


Eluting:
solution C

Pre-treatment by column I

Measurement of 8-OHdG

Measurement of 8-OHdG by a combination of general HPLC system and electrochemical detector



**→ Measurement time: 40 min
(Column switching method: 50 min)**

Recommend HPLC system

	Urine sample	Serum sample
HPLC system	LC-2010 series (SHIMAZU), LC-8020 series (TOSO) and so on. (Ordinary HPLC system)	LC-2010 series (SHIMAZU), LC-8020 series (TOSO) and so on. (Ordinary HPLC system)
Electro-chemical detector	Coulochem III (esa), NANOSPACE 3005 (SHISEIDO), ECD-700 (EiCOM), ED-703 (GL science) and so on	Coulochem III (esa)
Analyzed column	Develosil C₃₀ (NOMURA)	Develosil C₃₀ (NOMURA)
Guard column	Develosil C₃₀ (NOMURA)	Develosil C₃₀ (NOMURA)