Human E-cadherin EIA Kit (precoated)Manual

Human E-cadherin EIA KIT (precoated)

An enzyme immunoassay kit
for the quantitative determination of
soluble human E-cadherin

For research use only. Not for use in diagnostic or therapeutic procedures.

Catalog Number. 40-831-160007
For 96 assays

Table of contents

Introduction

E-cadherin, also known as uvomorulin or Cell-CAM120/80, is one of the subclasses of cadherins, Ca^{2+}–dependent cell adhesion molecules(1,2), found in epithelial cells in a variety of embryonic and adult tissues (3,4,5). Previous studies suggested that unstable or reduced expression of E-cadherin seems to be a common event in cancer progression, such as in lung carcinoma(6), gastric tumours(7), hepatocellular carcinoma(8), breast carcinoma(9,17), and prostatic tumours(10,11). Soluble forms of E-cadherin, 80~84 kDa protein, firstly found in the serum-free culture medium released from MCF-7 human carcinoma cells or artificially generated by trypsinising cells in the presence of calcium (3,4) and is presumed to be a degradation product of the 120 kDa form of intact E-cadherin generated by a Ca^{2+}–dependent proteolytic action(1,12). Recently, the 80 kDa form of soluble E-cadherin was found to be circulating in biological fluids of healthy persons(14), and was shown that the serum levels were elevated in the tumour patients(13,15) and those with systemic inflammatory response syndrome and multiorgan dysfunction syndrome(16).

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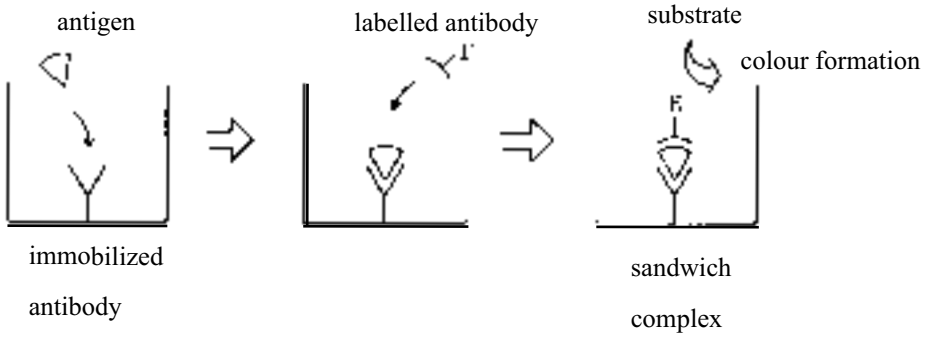
Intended use

The human E-cadherin EIA Kit is an *in vitro* enzyme immunoassay (EIA) kit for quantitative determination of soluble human E-cadherin in serum, urine, cell culture supernatants, and other biological fluids. This kit is suitable for quantitation of soluble human E-cadherin.

This kit is for research use only. It is not for use in diagnostic or therapeutic procedures.

Principle

The human E-cadherin EIA Kit is a solid phase EIA based on a sandwich method that utilizes two mouse monoclonal anti-human E-cadherin antibodies to detect soluble E-cadherin by two-step procedure. One of the mouse monoclonal anti-human E-cadherin is bound to a microtiter plate to create the solid phase. Non-specific binding is blocked by a blocking buffer. Samples and standards are incubated in microtiter-plate wells. The second step is to wash the plate and add a second anti-human E-cadherin labelled with peroxidase (POD). During the incubation, human E-cadherin is bound to anti-human E-cadherin (solid phase) on one side and tagged on the other by POD-anti-human E-cadherin. The reaction between POD and substrate (H_2O_2 and tetramethylbenzidine) results in colour development with intensities proportional to the amount of human E-cadherin present in samples and standards. The amount of human E-cadherin can be quantitated by measuring the absorbance using an EIA plate reader. Accurate sample concentrations of human E-cadherin can be determined by comparing their specific absorbances with those obtained for the standards plotted on a standard curve.



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Reagents and materials

Each human E-cadherin EIA Kit includes reagents sufficient for 96 wells. The expiration date for the complete kit is stated on the outer box label and the recommended storage temperature is 2 - 8°C.

A. Materials provided

Plate 1. Antibody Coated Microtiterplate - 1 plate (8 well x 12 strips)
The plate coated with murine monoclonal antibody to human E-cadherin. Store at 2 - 8°C.

Vial 2. Antibody-POD Conjugate - 1 vial (for 11 ml x 1)
The vial contains lyophilized horseradish peroxidase (POD) conjugated murine monoclonal antibody to human E-cadherin. Store at 2 - 8°C. Avoid prolonged exposure to light.

Vial 3. Standard - 1 vial (2700 ng; for 1 ml x 1)
The vial contains lyophilized human E-cadherin.

Vial 4. Sample Diluent - 2 vials (11 ml x 2)
Each vial contains protein in a buffered solution. Use for Zero standard, and for dilution of the standard (vial 3) and samples which are above the calibration curve. Store at 2 - 8°C.

Vial 5. Substrate Solution - 1 vial (12 ml x 1)
The vial contains hydrogen peroxide and tetramethylbenzidine in a buffered solution. Store at 2 - 8°C.

B. Materials required but not provided

1.Reagents

- Stop Solution: 1N H_2SO_4
- Washing Buffer: Tris-Buffered Saline (TBS) containing 0.1% Tween 20 and 5 mM $CaCl_2$
  (Tris-Buffered Saline (TBS) powder is convenient for the preparation of Washing Buffer.)
- Cell Extraction Buffer (In case of using cell culture):
  20mM Tris-HCl, pH7.5 containing 150 mM NaCl, 1 % NP-40, 5mM $CaCl_2$, and 1 mM PMSF

2.Materials

- Precision pipettes with disposable tips: 20 and 100 µl micropipettes,
- 10 - 200 µl adjustable multiwell pipetter or 20 µl and 100 µl multiwell pipetters
- Beakers, flasks, cylinders necessary for preparation of reagents
- Disposable pipettes and test tubes
- Microtiter plate reader for measurement of absorbance at 450 nm
- Graph paper

Precautions

- Do not mix reagents from different kit lots.

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- Do not use reagents beyond expiration date on label.
- In order to avoid reagent contamination, use disposable pipette tips and/or pipettes.
- Sodium azide inactivates POD. Solutions containing sodium azide should not be used in this assay.
- Do not expose Substrate Solution to strong light during storage or incubation.
- Avoid contact of Substrate Solution and Stop Solution with skin or mucous membranes. If these reagents come into contact with skin, wash thoroughly with water. Do not pipette by mouth. Do not smoke, eat, or drink in area where specimens or kit reagents are handled. All blood fluids should be considered as potentially infectious.
- Avoid contact of Substrate Solution and Stop Solution with any metal surfaces. Disposable glassware or test tubes are recommended for, handling the Substrate Solution. If non-disposable glassware is used, it must be acid washed and thoroughly rinsed with distilled, deionized water.
- Do not use the Substrate Solution if its colour is changed to thick blue.

Specimen collection and handling

Venous blood samples are collected aseptically. EDTA, or citrated plasma is not suitable for use in the assay. Serum, urine or cell culture supernatant can be used. Remove the serum from the clot or red cells soon after clotting and separation. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples may be stored up to 24 hours at 4°C. If the length of time between sample collection and assay is to exceed 24 hours, samples should be stored frozen under -20°C for optimal results. Excessive freeze-thaw cycles should be avoided. Prior to assay, frozen samples should be brought to room temperature slowly, and gently mixed by hand. Do not thaw samples in a hot bath. Do not vortex or sharply agitate. Do not use any chelating agents for Ca^{2+} ion, such as EDTA, for the preparation of samples. Insoluble form of E-cadherin is not detectable in this system.

Recommended Sample Dilution

In case of using plasma, serum, or urine, dilute the samples by about 10 folds before assay. When the diluted samples generate values out of the standard range, dilute the samples with the different dilution rate referring to the first assay result, and repeat the assay. Or it is recommended to assay using three kinds of sample dilutions making the 10 folds for plasma, serum, or urine as the middle concentration.

In case of using cell culture, add Cell Exraction Buffer to have the concentration of 5×10^6 - 1×10^7 cells / ml. Centrifuge, and use the supernatant as sample.

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Preparation of solutions

Note: The following solutions should be prepared directly before use.

Solution 1. Antibody-POD Conjugate Solution
Dissolve the contents of Vial 2 in 11 ml of distilled water and mix gently followed by 10 minutes slowly rolling or occasional mixing, avoiding foam formation.

Solution 2. Standard Solution
Rehydrate Standard (Vial 3) with 1 ml of distilled water. Slowly roll for approximately 10 minutes or let vials to stand and sporadically mix gently.
The standard solution contains approximately 2700 ng human E-cadherin/ml.
Prepare a dilution series of 1350, 675, 337.5, 168.8, 84.4 ng/ml by diluting the Standard Solution with Sample Diluent.

Stability of solutions

Solution 1. Antibody-POD Conjugate Solution
The reconstituted lyophilisate is stable for 1 week at 4°C and for 1 month when stored at -20°C. Do not repeat freeze-thaw cycles.

Solution 2. Standard Solution
The reconstituted lyophilisate is stable for 1 month stored at -20°C. Do not repeat freeze-thaw cycles.

Procedure

Double determinations of all samples and standards should be performed.
All of the Kit's content should be brought to room temperature before use !
For thorough mixing, the microtiter plate can be gently agitated on a plate mixer or by mixing the plate sporadically by hand.

-
[Enzyme immunoassay]

Sample incubation: Pipette 100 µl of sample or standard (Solution 2) into one well within 5 minutes. Mix, seal the microtiter plate (e.g. with a foil) and incubate for 2 hours at 37°C.

- Remove sample solution and wash the wells 3 times with ca. 400 µl of
- Washing Buffer; between the separate washing steps, empty out the microtiter plate and vigorously tap onto paper towel, especially after the last washing.
Antibody-POD conjugate incubation: Pipette 100 µl of Antibody-POD Conjugate Solution (Solution 1) into one well, mix and seal the microtiter plate (e.g. with a foil) and incubate 1 hour at 37°C.
- Remove sample solution and wash the wells 4 times as described above (It is
- specially important after this step to thoroughly empty out the remaining fluid before adding the substrate).
- *Substrate incubation:* Add 100 µl of Substrate Solution (Vial 5) into each well and incubate at room temperature (20 - 30°C) for 15 minutes.
- Add 100 µl of Stop Solution (1N H_2SO_4) into each well in the same order as for substrate. Tap plate gently to mix.
Measure the absorbance at 450 nm with a plate reader. The absorbance should be read as soon as possible after the completion of the assay. It may be read up to 1 hour after addition of Stop Solution if wells are protected from light at room temperature.

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Note: It is important that Stop Solution is added to wells prior to reading at 450 nm. Addition of Stop Solution causes an increase in absorbance of the Substrate Solution and shift in absorbance spectrum.

Results

1. Standard curve
- Record the absorbance at 450 nm for each standard well.
- Average the duplicate values and record the averages.
- Plot the absorbance (vertical axis) versus the E-cadherin concentration in ng/ml (horizontal axis) for the standards using optimal fitting curve or log-log scale.

2. Samples
- Record the absorbance at 450 nm for each sample well.
- Average the duplicate values and record the averages.
- Locate the average absorbance value on the vertical axis and follow a horizontal line intersecting the standard curve. At the point of intersection, read the E-cadherin concentration (ng/ml) from the horizontal axis.

Performance characteristics

1. **Range of standard curve**: 84.4 - 2700 ng/ml.
2. **Specificity:** This kit specifically measures soluble human E-cadherin. This kit cannot be used to measure mouse E-cadherin. The application of this kit for quantitating E-cadherin from other sources has not been tested.
3. **Assay duration:** Three and a half hours after sample incubation.
4. **Total assay capacity:** 96 assays.
5. **Assay capacity for test samples:** If all assay wells (including standards and test samples) are run in duplicate, 40 test samples can be run in duplicate per kit.
6. **Test specimen type:** Human serum or urine; culture supernatants.
7. **Specimen volume required:** If each test sample is run in duplicate, approximately 220 µl (i.e., 100 µl per assay well plus ~10 µl for each sample transfer) is required.
8. **Limitation:** Since conditions may vary from assay to assay, a standard curve must be established for every run. Since cross contamination between reagents will invalidate the test, disposable pipette tips should be used.

   Thorough washing of the wells between incubations is required:
   1) Completely empty out the remaining fluid from the well before dispensing fresh wash solution.
   2) Use sufficient wash solution for each wash cycle (approximately 400 µl).
   3) Do not allow wells to sit uncovered for extended periods between incubation steps.

   Only samples with absorbance values falling within the range of the standard curve should be assigned a human E-cadherin concentration from the curve.

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9. **Notes :** According to the assay results using control serum or urine, it could be possible to determine the concentration of antigen present in a biological fluid. Linear dilution curves were obtained in the measurements for serum E-cadherin (13). However, the measurement may be potentially disturbed by the unknown organic factors in serum or urine samples in patients with specific diseases. Similarly, a specimen obtained from an apparent healthy subject might also be interrupted. When an antigen level in an unknown organic specimen is observed to be elevated as compared to the calibration range of the standard curve, it is recommended to dilute the specimens properly with the dilution solution included in the kit and assay them again in another run.

Basal data

1. Typical Standard Curve

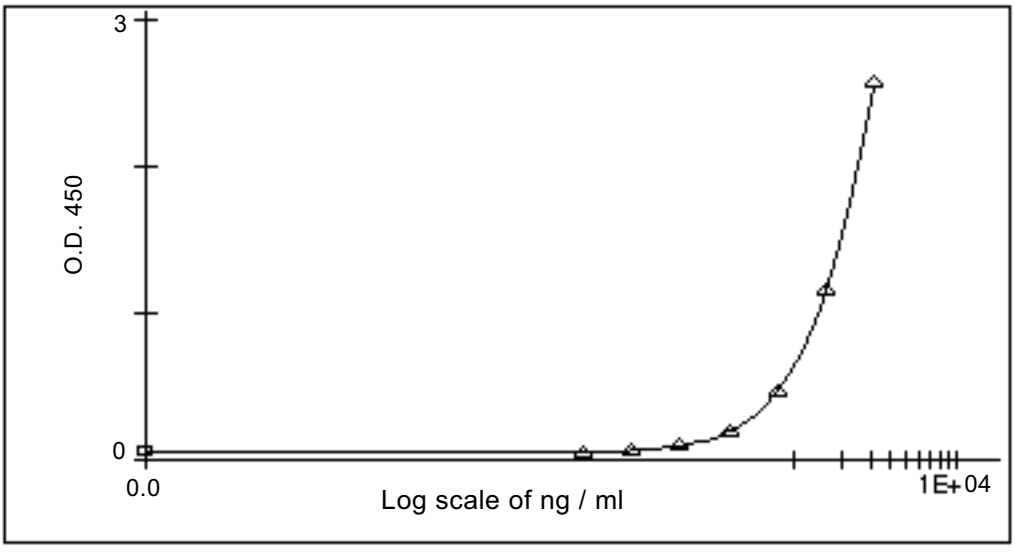
Each laboratory should establish its own normal range for soluble E-cadherin level.

(Do Not Use To Calculate Unknowns)

Curve Fit: 4-Parameter Corr. Coeff: -1.00

$y = (A-D) / [1 + (x / C)^B] + D$

A = 0.0696 B = 1.81 C = 1.27E + 03 D = 3.29



E-cadherin (ng / ml)	2700	1350	675.0	337.5	168.8	84.4	42.2	0
A_{450}	2.635	1.765	0.854	0.324	0.158	0.101	0.079	0.060

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2. **Intra-assay precision (n=16)**

Assay was carried out with 16 replicates of 3 samples containing different concentration of control soluble E-cadherin.

Sample	Ave. (ng/ml)	S.D. (ng/ml)	CV (%)
Control A	1935.0	179.5	9.3
Control B	606.9	41.7	6.9
Control C	157.2	10.9	6.9

Inter-assay precision (performance 3 times)

Assay to assay precision with one laboratory was evaluated in three independent experiments over 3 days.

Sample	Ave. (ng/ml)	S.D.(ng/ml)	CV (%)
Control A	1944.0	99.9	5.1
Control B	637.0	26.1	4.1
Control C	152.8	7.1	4.6

3. Recovery test

The recovery of E-cadherin was tested by adding two samples out of five different level in various matrices.

(unit : ng/ml)

Sample A	Sample B	A+B Measured	A+B Calculated	Recovery (%)
2075.0	0.0	1007.0	1038.0	97.1
2075.0	2075.0	2391.0	2075.0	115.2
2075.0	1178.0	1834.0	1627.0	112.8
2075.0	644.6	1552.0	1360.0	114.1
2075.0	363.5	1395.0	1219.0	114.4
2075.0	180.9	1222.0	1128.0	108.3
1178.0	0.0	554.5	589.0	94.1
1178.0	1178.0	1074.0	1178.0	91.2
1178.0	644.6	980.1	911.3	107.5
1178.0	363.5	768.0	770.8	99.6
1178.0	180.9	722.1	679.5	106.3
644.6	0.0	307.8	322.3	95.5
644.6	644.6	550.6	644.6	85.4
644.6	363.5	467.0	504.1	92.6
644.6	180.9	371.6	412.8	90.0
363.5	0.0	192.1	181.8	105.7
363.5	363.5	338.2	363.5	93.0
363.5	180.9	265.2	272.2	97.4
180.9	0.0	126.7	90.5	140.1
180.9	180.9	173.1	180.9	95.7

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4. Daily variation of urinary E-cadherin excresion

The daily variation of urinary E-cadherin value was measured using the samples from 3 individuals.

-The samples were used after 9-fold dilution. The following table shows the estimated E-cadherin values at the original concentration. The samples were collected freely during daytime. (This data was obatined by using a former kit)

	Sampling Date	E-cadherin (μg/ml)	Creatinin (Cr) (mg/ml)	E-cadherin/Cr (mg/g)
Female 1	day 1	3.566	1.264	2.82
	day 2	3.671	1.004	3.66
	day 3	2.620	0.605	4.33
	day 4	4.080	1.514	2.69
	day 5	8.035	2.346	3.42
	day 6	8.046	1.915	4.20
	day 7	5.106	1.313	3.89
	day 8	2.936	0.483	6.08
Female 2	day 1	1.311	1.182	1.11
	day 2	1.216	2.045	0.59
	day 3	0.776	1.594	0.49
	day 4	0.313	1.565	0.20
	day 5	0.729	1.814	0.40
	day 6	0.568	2.562	0.22
	day 7	0.467	2.031	0.23
	day 8	0.514	1.579	0.33
Male 3	day 1	0.855	1.195	0.73
	day 2	0.067	0.403	0.17
	day 3	0.876	1.016	0.86
	day 4	1.299	1.397	0.93
	day 5	0.577	0.422	1.37
	day 6	0.770	0.561	0.14
	day 7	1.719	1.466	1.17
	day 8	1.036	0.645	1.61

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5. Urinary E-cadherin excresion in a day

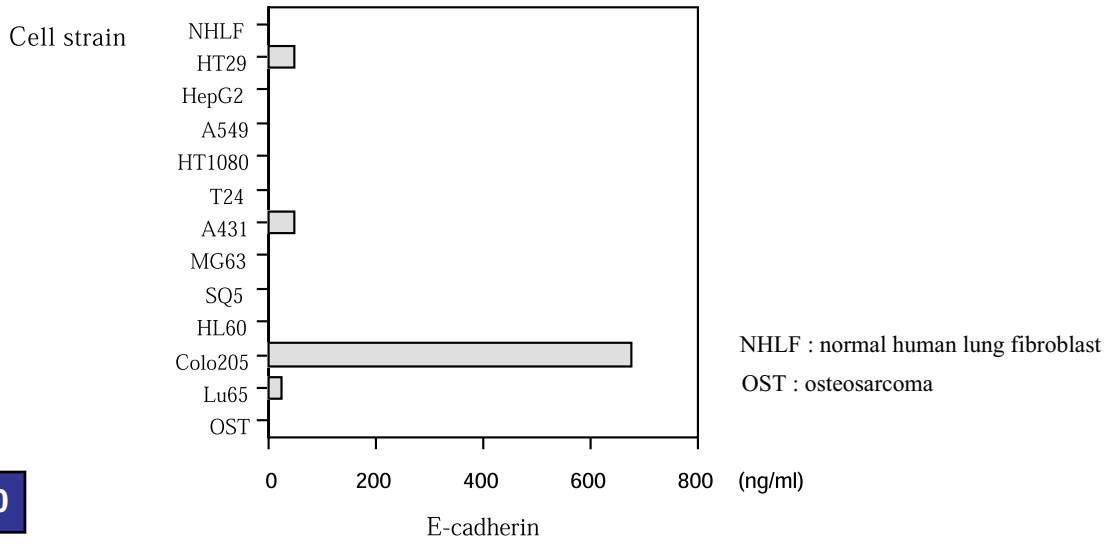
The amount of urinary E-cadherin excresion in a day was measured with the samples collected from 4 individuals.

-The samples were used after 9-fold dilution. The following table shows the estimated E-cadherin values at the original concentration. The samples were collected freely during a day. (This data was obatined by using a former kit)

	Urine (ml)	E-cadherin (μg/ml)	Creatinin (Cr) (mg/ml)	E-cadherin/Cr (mg/g)
Male 2	270	0.699	1.760	0.40
	525	0.286	1.100	0.26
	70	1.554	1.489	1.04
	570	0.421	0.897	0.47
Female 3	80	1.246	1.311	0.94
	100	1.146	1.441	0.80
	180	0.855	1.478	0.58
	200	1.485	0.974	1.52
	180	0.928	1.466	0.63
Male 3	350	0.494	0.584	0.85
	470	0.634	0.481	1.32
	370	0.359	0.676	0.53
	180	0.778	1.548	0.50
	130	0.592	1.890	0.31
Male 4	250	1.789	1.718	1.04
	150	4.043	2.759	1.47
	160	3.837	1.579	2.43
	175	3.442	2.400	1.43
	175	2.453	1.844	1.33

6. E-cadherin in cell supernatant

The amount of E-cadherin in the supernatant of various cells cultured in 10% FCS/ RPMI1640) was measured. The supernatant was applied to assay without dilution. (This data was obatined by using the precoated kit #40-831-160007.)



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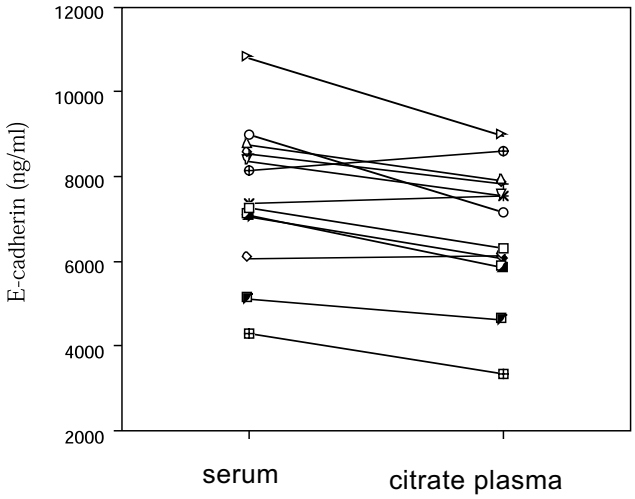
7. Comparison between citrate plasma and serum value of E-cadherin

The blood samples were collected from the same individual. There is no significant difference between serum and citrate plasma E-cadherin level. Normal value should be determined with statistically adequate number of samples (n=13).

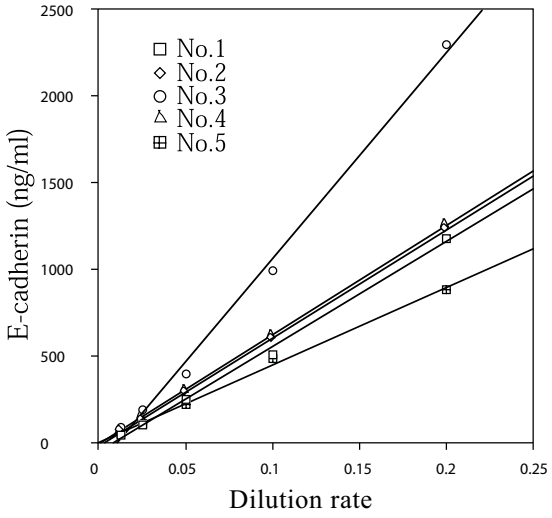
Normal value of E-cadherin



8. Dilution curves of serum and urine samples

Each assay was performed using 5 kinds of sample which were prepared as dilution series starting from 5-fold.

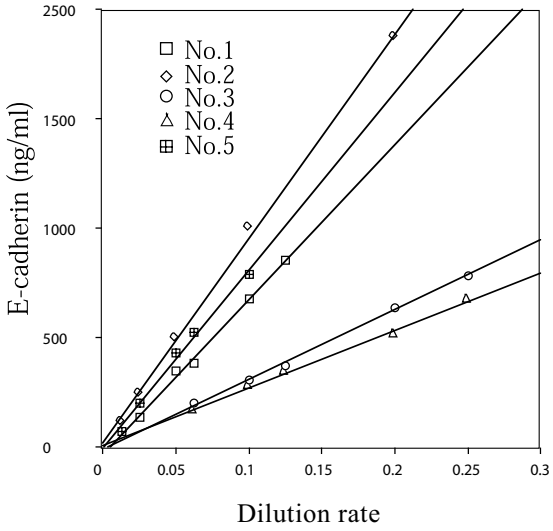
Serum



Curve Fit: 4-Parameter Corr.

y = 6051.720x - 49.308 r = 0.998 No.1
y = 6245.355x - 19.475 r = 1.000 No.2
y = 11903.172x - 125.096 r = 0.997 No.3
y = 6308.333x - 6.196 r = 1.000 No.4
y = 4450.892x + 6.196 r = 0.998 No.5

Urine



Curve Fit: 4-Parameter Corr.

y = 7096.235x - 36.297 r = 0.998 No.1
y = 9362.204x + 15.529 r = 0.999 No.2
y = 3178.591x - 10.362 r = 0.999 No.3
y = 2611.473x + 6.348 r = 0.998 No.4
y = 8138.667x - 6.073 r = 0.996 No.5

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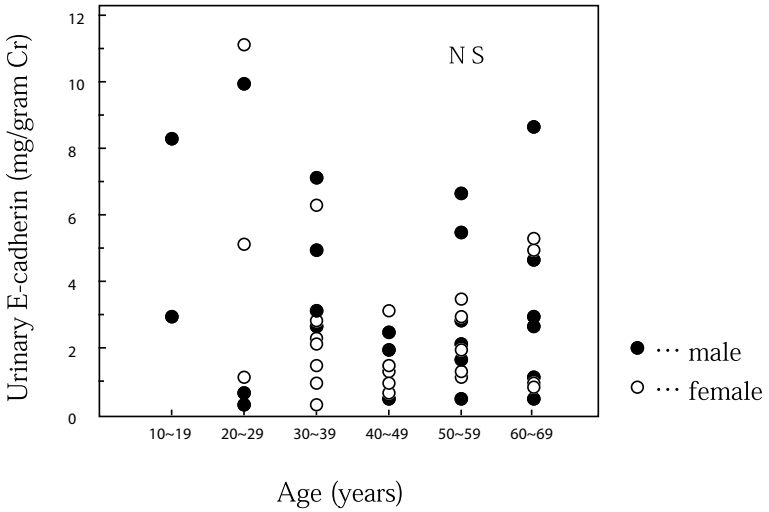
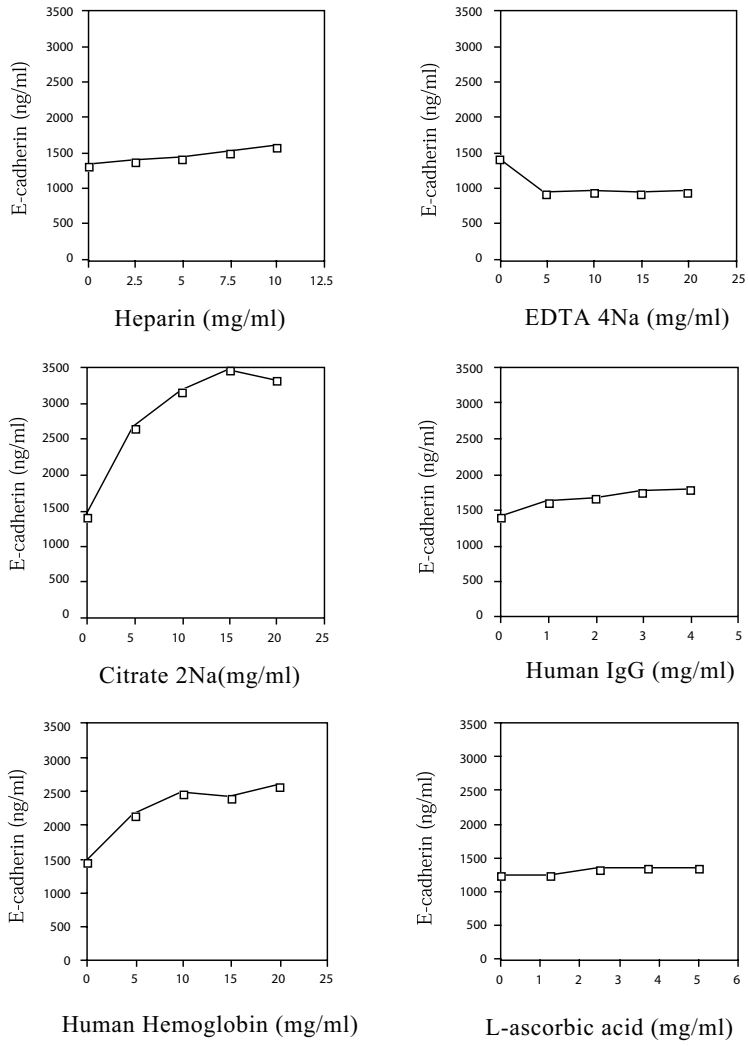
9. Alteration of urinary E-cadherin value in aging (14)

There is no significant correlation between urinary E-cadherin value in urine and aging.



10. Influence of coexistence

The volume ratio of sample to co-existing substance is 4:1. Co-existing substance is shown in its final concentration.



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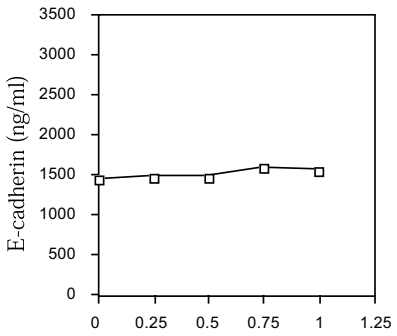
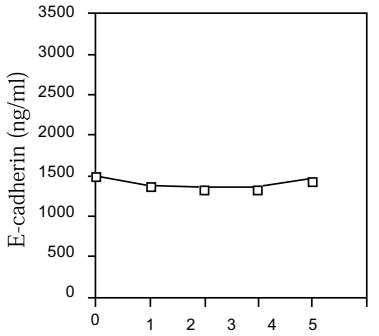
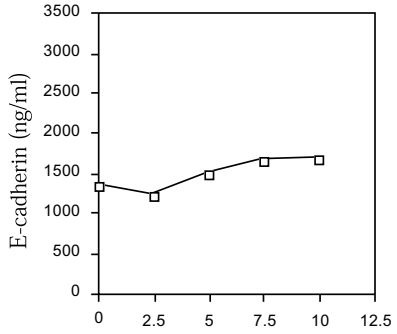


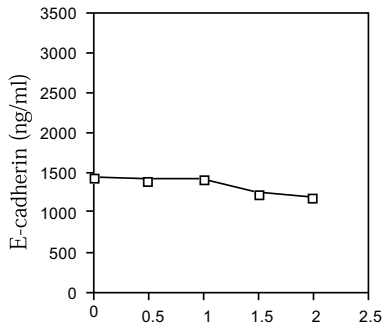
Human Fibrinogen (mg/ml)



Human Albumin (mg/ml)



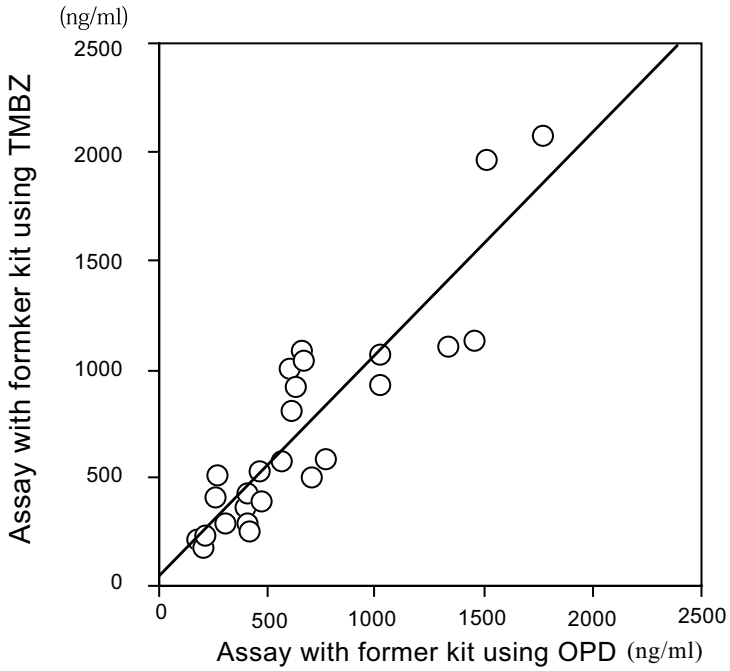
Calcium Chloride (mg/ml)



Bilirubin(mg/ml)

11. Correlation with the former kit

Correlation of precoated type kit assay to that of the former kit. The former kit employed *O*-phenylenediamine (OPD) as the substrate, and the precoated one employs 3,3',5,5'-tetramethylbenzidine (TMBZ) as the substrate.



y = 1.026x + 46.108
r = 0.899
n = 26

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Storage and stability

This kit is shipped at 2 - 8°C and should be stored at 2 - 8°C if not used. Under this condition, the kit is stable until expiration date on label.

References

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Protocol summary

1. Prepare all reagents as directed in the Package Insert.
2. Bring all reagents to room temperature and prepare the solutions.
3. Add 100 µl of Standard or sample to appropriate wells, and incubate for 2 hours at 37°C.
4. Remove sample solution and wash the wells 3 times with ca. 400 µl of Washing Buffer.
5. Add 100 µl of antibody-POD conjugate solution into wells and incubate at 37°C for 1 hour.
6. Aspirate solution from wells. Wash 4 times with ca. 400 µl of Washing Buffer per wells, aspirating thoroughly between washes.
7. Add 100 µl of Substrate Solution to each well. Incubate 15 minutes at room temperature.
8. Add 100 µl of Stop Solution to all wells. Mix gently.
9. Read at 450 nm as soon as possible.

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