YK180　Estrone EIA

(For measurement of environmental water and culture medium supernatant)

FOR LABORATORY USE ONLY

YANAIHARA INSTITUTE INC.
2480 -1 AWAKURA, FUJINOMIYA - SHI
SHIZUOKA, JAPAN 418 - 0011

Contents

- Please read all the package insert carefully before beginning the assay -

YK180 Estrone EIA Kit

（For measurement of estrone in environmental water and culture medium supernatant）

Ⅰ. Introduction

Estrone is a member of estrogen, which is now attracting public attention as an environmental pollutant, especially in rivers and wastewater. Yanaihara Institute Inc. developed a quantitative EIA kit with high specificity and sensitivity for estrone in environmental water. This assay kit is proved to have crossreactivity with neither testosterone nor androstenedione.

More practically, this estrone EIA kit can be used in assessment of endocrine disrupting effects of environmental contaminants and chemicals used in commercial products. In brief, using a human ovarian granulose-like tumor cell line with high aromatase activity and substantially no synthetic activity of androgen and estrogen, the effect of a test compound on the aromatase, a key enzyme in the conversion of androgens to estrogens, is assessed by measuring estrone in culture medium produced from androstenedione added. Reduced concentration of estrone in the medium screens compounds that can disrupt endocrine function by influencing aromatase activity. The usefulness of our estrone specific EIA kit was fully established for this purpose.

YK180 Estrone EIA Kit

▼ The assay kit can measure estrone within the range of 4.8 - 5,000pg/mL.

▼ The assay is completed within 17-19 hr.＋4 hr.

▼ With one assay kit, 41 samples can be measured in duplicate.

▼ Test sample: Environmental water and culture medium supernatant
Sample volume: 100 μL

▼ The 96-wells plate in kit is consisted by 8-wells strips, and the strips can be used separately.

▼ Precision and reproducibility
Environmental water
Intra-assay CV (%) 5.75 - 15.99
Inter-assay CV (%) 5.98 - 14.50
Culture medium supernatant
Intra-assay CV (%) 3.70 - 15.59
Inter-assay CV (%) 4.90 - 19.69

▼ Stability and Storage
Store all of the components at 2-8ºC.
The kit is stable under the condition for 24 months from the date of manufacturing.
The expiry date is stated on the label of kit.

Contents

1) Antibody coated plate
2) Standard
3) Labeled antigen
4) Specific antibody
5) SA-HRP solution
6) Substrate buffer
7) OPD tablet
8) Stopping solution
9) Buffer solution
10) Washing solution (concentrated)
11) Adhesive foil

Ⅱ. Characteristics

This EIA kit is used for quantitative determination of estrone in environmental water and culture medium supernatant. The kit is characterized by its sensitive quantification and high specificity. In addition, it has no influence by other components in culture medium supernatant.

＜Specificity＞

This EIA kit shows the following crossreactivities: 100% to estrone, 30.6% to 17β- estradiol and 0.4% to estriol. The crossreactivity to testosterone, progesterone, 4-androstene-3, 17-dione and cholesterol are less than 0.0049%.

＜Assay principle＞

This EIA kit for determination of estrone in environmental water and culture supernatant sample is based on a competitive enzyme immunoassay using combination of highly specific antibody to estrone and biotin-avidin affinity system. To the wells of plate coated with goat anti rabbit IgG antibody, labeled antigen (biotinylated antigen), standards or samples and rabbit anti estrone antibody are added for competitive immunoreaction. After incubation and plate washing, horseradish peroxidase (HRP) labeled streptoavidin (SA) is added to form HRP labeled SA-labeled antigen-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by o-Phenylenediamine dihydrochloride (OPD) and the concentration of estrone is calculated.

Ⅲ. Composition

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	microtiter plate	1 plate (96 wells)	Goat anti rabbit IgG coated
2.	Standard	lyophilized*	1 vial (400ng)	Estrone
3.	Labeled antigen	lyophilized	1 vial	Biotinylated estrone
4.	Specific antibody	lyophilized	1 vial	Rabbit anti estrone antibody
5.	SA-HRP solution	liquid	1 bottle (12mL)	Horseradish peroxidase labeled streptoavidin
6.	Substrate buffer	liquid	1 bottle (24 mL)	0.015% Hydrogen peroxide
7.	OPD tablet	tablet	2 tablets	o-Phenylenediamine dihydrochloride
8.	Stopping solution	liquid	1 bottle (12 mL)	1M H_2SO_4
9.	Buffer solution	liquid	1 bottle (25 mL)	Phosphate buffer
10.	Washing solution (concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
11.	Adhesive foil		3 pieces	

* Standard in the vial is invisible to the naked eye due to small quantities.

Ⅳ. Method

<Equipment required>

1. Photometer for microtiter plate (plate reader) , which can read extinction 2.5 at 490 nm (or 492 nm)
2. Microtiter plate shaker
3. Washing device for microtiter plate and dispenser with aspiration system
4. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
5. Glass test tubes for preparation of standard solution
6. Graduated cylinder (1,000 mL)
7. Distilled water or deionized water

<Preparatory work>

1. Preparation of standard solution :

   Reconstitute the estrone standard (lyophilized 400 ng/vial) with 1 mL of 70% ethanol (not including in this kit), which affords 400 ng/mL standard solution. The 0.1 mL of the reconstituted standard solution is diluted with 7.9 mL of buffer solution that yields 5,000 pg/mL standard solution. The 0.2 mL of the 5,000 pg/mL standard solution is diluted with 0.6 mL of buffer solution that yields 1,250 pg/mL standard solution. Repeat the dilution procedure to make each standard solution of 312.5, 78.1, 19.5 and 4.8 pg/mL. Buffer solution is used as 0 pg/mL standard solution.

   If a sample concentration below 4.8 pg/mL is predicted, standard curve may be further set up a lower detection limit by using 1.2 pg/mL standard solution which can be prepared by 4-fold dilution of 4.8 pg/mL standard solution. In such case, however, assay precision may not be so excellent as that of the cases between 4.8 and 5,000 pg/mL.
2. Preparation of labeled antigen :

   Reconstitute labeled antigen with 6 mL of distilled or deionized water.
3. Preparation of specific antibody :

   Reconstitute specific antibody with 6 mL of distilled or deionized water.
4. Preparation of substrate solution :

   Dissolve one OPD tablet with 11 mL of substrate buffer. It should be prepared immediately before use.
5. Preparation of washing solution :

   Dilute 50 mL of washing solution (concentrated) to 1,000 mL with distilled or deionized water.
6. Other reagents are ready for use.

<Procedure>

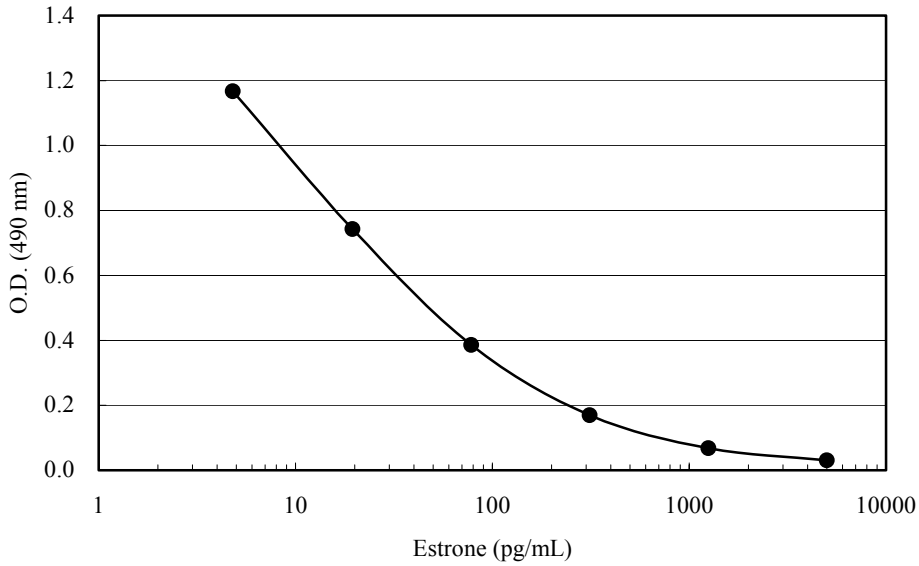
1. Before starting the assay, bring all the reagents and samples to room temperature (20 ~ 30ºC).
2. Fill 0.35mL/well of washing solution into the wells and aspirate the washing solution in the wells. Repeat this washing procedure further twice (total 3 times). Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
3. Add 50 μL of labeled antigen to the wells first, then introduce 100 μL of each of standard solutions (0, 4.8, 19.5, 78.1, 312.5, 1,250 and 5,000 pg/mL) or samples, and finally add 50 μL of specific antibody to the wells.
4. Cover the plate with adhesive foil and incubate it at 4ºC for 17-19 hours (keep still, plate shaker not need).
5. After incubation, move the plate back to room temperature keeping for approximately 60 minutes (keep still, plate shaker not need) and take off the adhesive foil, aspirate and wash the wells 4 times with 0.35 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
6. Add 100 μL of SA-HRP solution to each of the wells.
7. Cover the plate with adhesive foil and incubate it at room temperature (20-30ºC) for 2 hours. During the incubation, the plate should be shaken with a plate shaker (approximately 100 rpm).
8. Dissolve one OPD tablet with 11 mL of substrate buffer. It should be prepared immediately before use.
9. Take off the adhesive foil, aspirate and wash the wells 4 times with 0.35 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
10. Add 100 μL of substrate solution to each of the wells, cover the plate with adhesive foil and keep it for 20 minutes at room temperature (keep still, plate shaker not need).
11. Add 100 μL of stopping solution to each of the wells to stop color reaction.
12. Read the optical absorbance of the wells at 490 nm (or 492 nm). The dose-response curve of this assay fits best to a 4 (or 5)-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4 (or 5)-parameter logistic function. Otherwise calculate mean absorbance values of wells containing standards and plot a standard curve on semi logarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this standard curve.

Ⅴ. Notes

1. Samples must be used as soon as possible after collection. If the samples are tested later, they should be divided into test tubes in small amount and frozen at or below -30ºC. Avoid repeated freezing and thawing of samples.
2. Standard, labeled antigen, specific antibody and substrate solutions should be prepared immediately before use. This kit can be used dividedly in strips of the plate. In such case, reconstituted standard solution, labeled antigen and specific antibody should be divided into test tubes in small amount and stored at or below -30ºC (stable for 1 month).
3. During storage of washing solution (concentrated) at 2-8ºC, precipitates may be observed, however they will be dissolved when diluted. Diluted washing solution is stable for 6 months at 2-8ºC.
4. Pipetting operation may affect the precision of the assay, so that pipette standard solutions or samples precisely into each well of plate. In addition, use clean test tubes or vessels in assay and use new tip for each standard or sample to avoid cross contamination.
5. When sample value exceeds 5,000 pg/mL, it needs to be diluted with buffer solution to proper concentration.
6. During the incubation with SA-HRP solution at room temperature, the assay plate should be shaken gently by a plate shaker to promote immunoreaction (approximately 100 rpm).
7. Perform all the determination in duplicate.
8. Read plate optical absorbance of reaction solution in wells as soon as possible after stop color reaction.
9. To quantitate accurately, always run a standard curve when testing samples.
10. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.
11. Satisfactory performance of the test is guaranteed only when reagents are used from combination pack with identical lot number.

VI. Performance Characteristics

Typical standard curve



< Analytical recovery >

Environmental water A

Added Estrone (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
0.0	4.08		
20.0	24.53	24.08	101.87
100.0	106.87	104.08	102.68
1000.0	904.85	1004.08	90.12

Environmental water B

Added Estrone (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
0.0	3.93		
20.0	24.49	23.93	102.34
100.0	93.56	103.93	90.02
1000.0	874.53	1003.93	87.11

Environmental water C

Added Estrone (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
0.0	2.86		
20.0	24.12	22.86	105.51
100.0	92.72	102.86	90.14
1000.0	890.71	1002.86	88.82

Culture medium supernatant A (Phenol red +)

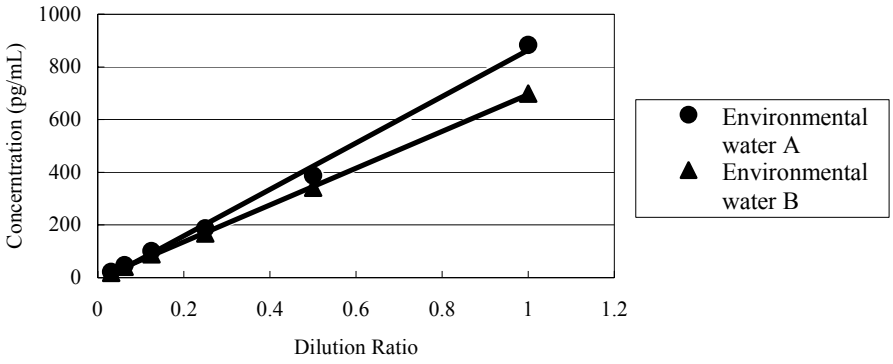
Added Estrone (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
0.0	2.03		
20.0	19.37	22.03	87.93
100.0	85.49	102.03	83.79
1000.0	855.09	1002.03	85.34

Culture medium supernatant B (Phenol red−)

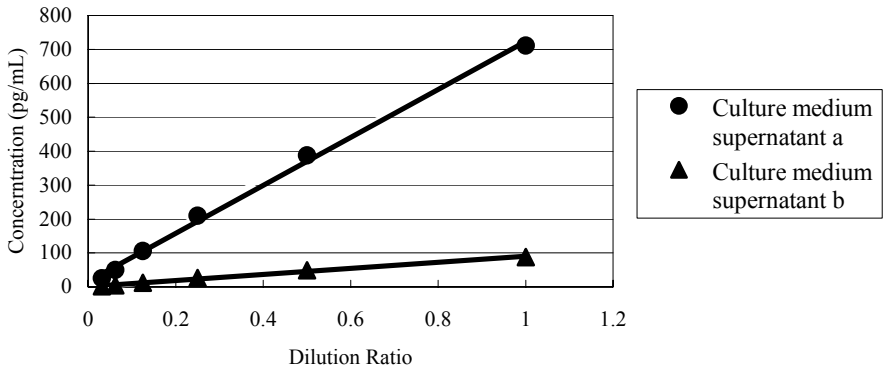
Added Estrone (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
0.0	1.78		
20.0	20.16	21.78	92.56
100.0	107.25	101.78	105.37
1000.0	983.85	1001.78	98.21

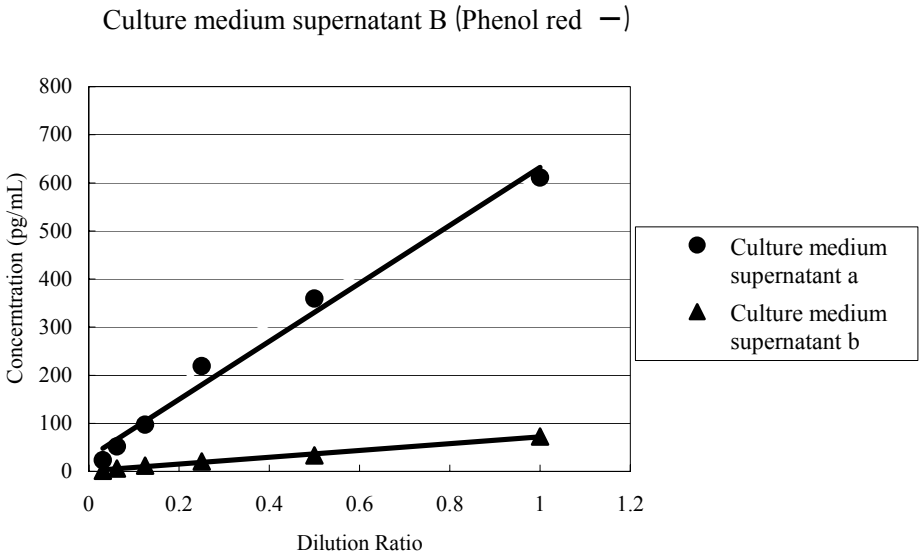
< Dilution test >

Environmental water



Culture medium supernatant A (Phenol red +)





<Crossreactivity>

Compounds	Crossreactivity (%)
Estrone	100
17β-estradiol	30.6
Estriol	0.4
Testosterone	< 0.0049
Progesterone	< 0.0049
4-androstene-3,17-dione	< 0.0049
Cholesterol	< 0.004

<Precision and reproducibility>

Test sample	Intra-assay CV(%)	Inter-assay CV(%)
Environmental water	5.75-15.99	5.98-14.50
Culture medium supernatant	3.70-15.59	4.90-19.69

<Assay range>

4.8-5,000 pg/mL

Ⅶ. Stability and Storage

< Storage > Store all of the components at 2-8ºC.

< Shelf life > The kit is stable under the condition for 24 months from the date of manufacturing.
The expiry date is stated on the label of kit.

< Package > For 96 tests per one kit including standards

Ⅷ. References

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<Manufacturer>
Yanaihara Institute Inc.
2480-1 Awakura, Fujinomiya-shi
Shizuoka, Japan 418-0011
TEL: +81-544-22-2771 FAX: +81-544-22-2770
Website: http://www.yanaihara.co.jp E-mail: ask@yanaihara.co.jp

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