COSMO BIO CO., LTD.
Inspiration for Life Science

Estrone EIA

(For measurement of environmental water and culture medium supernatant)

Cat. No. YII-YK180-EX

FOR LABORATORY USE ONLY

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

YII-YK180-EX: 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 in the range of 4.8 - 5,000pg/mL.

▼ The assay completes 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 was 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
24 months from the date of manufacturing.
The expiry date is described on the label of kit.
Store all of the components at 2-8°C.

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 for sensitive quantification, high specificity and no influence with other components in culture medium supernatant.

＜Specificity＞

The 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%.

＜Test 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. The 96-wells plate is coated with goat anti rabbit IgG. Biotinylated estrone, estrone standard or samples and rabbit anti estrone antibody are added to the wells for competitive immunoreaction. After incubation and plate washing, HRP labeled streptoavidin (SA-HRP) are added to form HRP labeled streptoavidin-biotinylated estrone-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	MTP[*1]	1 plate (96 wells)	Goat anti rabbit IgG
2. Standard	lyophilized[*2]	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 (12 mL)	HRP 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 sheets	

MTP[*1]. . . . . Microtiter plate

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

Ⅳ. Method

<Equipment required>

1. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
2. Photometer for microtiter plate (Plate reader), which can read extinction 2.5 at 492 nm
3. Microtiter plate shaker
4. Test tubes for preparation of standard solution
5. Washing device for microtiter plate and dispenser with aspiration system
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 same dilution to make each standard of 312.5, 78.1, 19.5, 4.8 pg/mL. Buffer solution is used as 0 pg/mL.

   <Assay range> 4.8-5,000 pg/mL

   If the sample value estimates below the 4.8 pg/mL, one more standard solution should be set up. It should be diluted 4.8 pg/mL standard solution to 1.2 pg/mL in this case, 40 samples can be measured in duplicate. Use the calculated sample value which is between the concentration of 1.2 pg/mL～4.8 pg/mL as an approximate value.
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

   Resolve 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 1000 mL with distilled or deionized

water.

6. Other reagents are ready for use.

<Procedure>

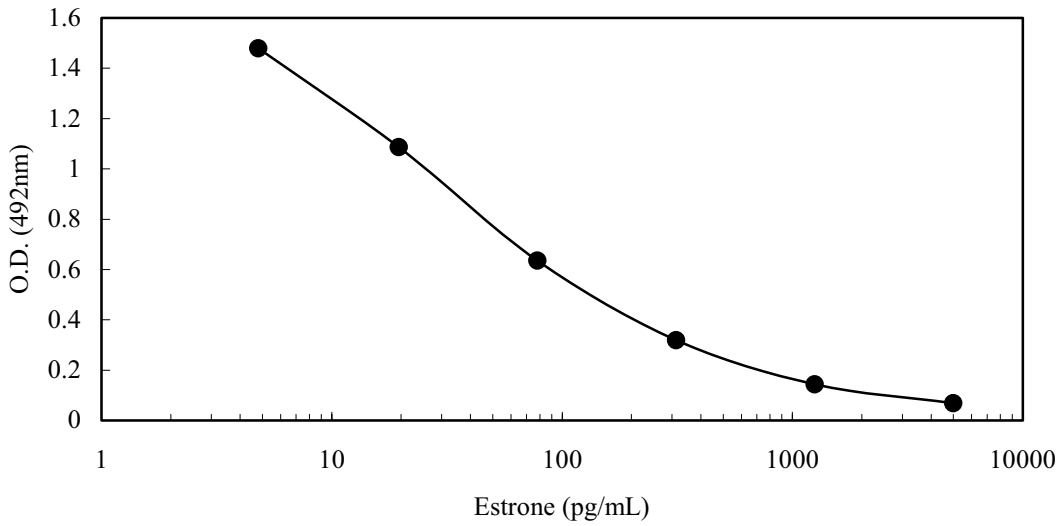
1. Bring all the reagents and samples to room temperature before beginning the test.
2. Add 0.35mL/well of washing solution into the wells and aspirate the washing solution in the wells. Repeat this washing procedure further twice (total 3 times). Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
3. Fill 50 µL of labeled antigen and add 100 µL of each of standard solutions (0, 4.8, 19.5, 78.1, 312.5, 1,250, 5,000 pg/mL) or samples, then introduce 50 µL of specific antibody into the wells.
4. Cover the plate with adhesive foil and incubate it at 4°C for 17-19 hours (Still, shaker not need)
5. After incubation, move the plate back to room temperature keeping for about 60 minutes and take off the adhesive foil, aspirate and wash the wells four times with approximately 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. Pipette 100 µL of SA-HRP solution into the wells.
7. Cover the plate with adhesive foil and incubate it at room temperature (20-30°C) for two hour. During the incubation, the plate should be shake with a plate shaker.
8. Resolve 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 four times with approximately 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 into the wells, cover the plate with adhesive foil and incubate it for 20 minutes at room temperature.
11. Add 100 µL of stopping solution into the wells to stop color reaction.
12. Read the optical absorbance of the wells at 492 nm. Calculate mean absorbance values of wells containing standards and plot a standard curve on semilogarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values). Use the standard curve to read estrone concentrations in samples from the corresponding absorbance values.

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 -30°C. Avoid repeated freezing and thawing of samples.
2. Estrone standard, labeled antigen, specific antibody and substrate solution should be prepared immediately before use. The plate can be used separately, in that case reconstituted standard solution, labeled antigen and specific antibody should be divided into test tubes in small amount and stored at or below -30°C.
3. During storage of washing solution (concentrated) at 2-8°C, precipitates may be observed, however they will be dissolved when diluted. Diluted washing solution is stable for 6 months at 2-8°C.
4. Pipetting operation may affect the precision of the assay, pipette precisely standard solutions or samples into each well of plate. Using clean test tubes or vessels in assay and use a new tip for each sample to avoid cross contamination.
5. When sample value exceeds 5,000 pg/mL, it needs to be diluted with buffered solution to proper concentration.
6. During incubation with SA-HRP solution at room temperature, the test plate should be shake gently by plate shaker to promote immunoreaction.
7. During continuous shake of test plate, the plate shaker may be heated up. It is recommended to place styrene form or plywood between the plate and the shaker.
8. Perform all the determination in duplicate.
9. Read plate optical absorbance of reaction solution in wells as soon as possible after stopping color reaction.
10. To quantitate accurately, always run a standard curve when testing samples.
11. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.
12. 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 supernatant A (Phenol red +)

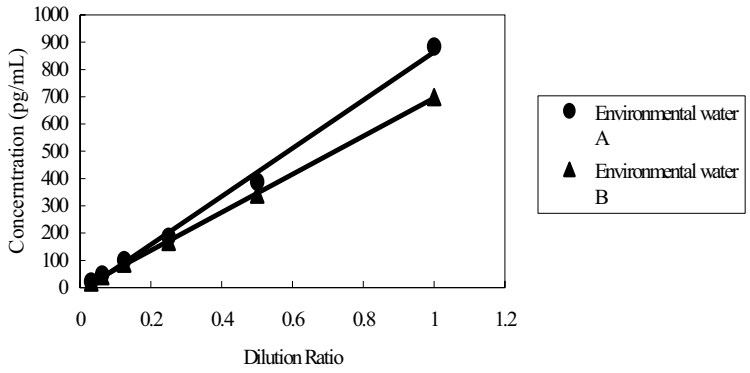
Added Estrone (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
0.0	2.03		
20.0	19.37	22.03	87.93
100.0	85.49	102.03	83.79
1000.0	855.09	1002.03	85.34

Culture supernatant B（Phenol red －）

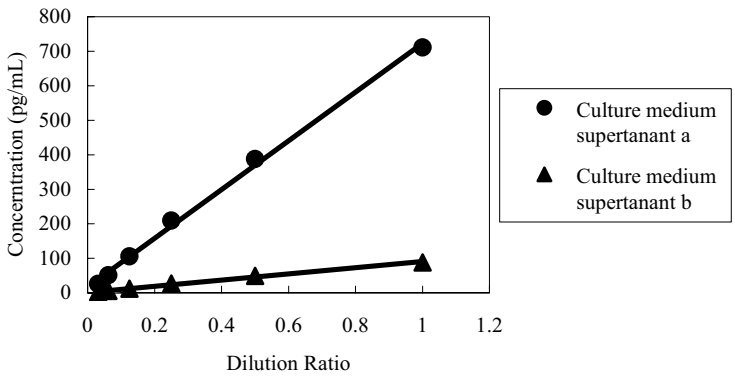
Added Estrone (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
0.0	1.78		
20.0	20.16	21.78	92.56
100.0	107.25	101.78	105.37
1000.0	983.85	1001.78	98.21

< Dilution Test >

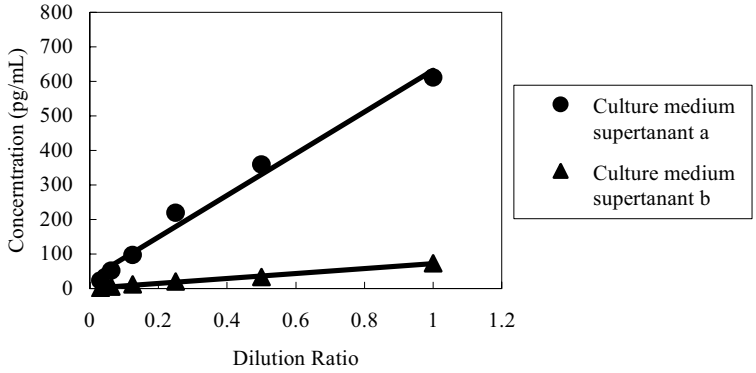
Environmental water



Culture medium supernatant A (Phenol red +)



Culture medium supernatant B（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>

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

Ⅶ. Stability and Storage

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

< Shelf life > 24 months from the date of manufacturing
The expiry date is described on the label of kit.

< Package > For 96 tests per one kit including standards

Ⅷ. References

1. Nakada N. et al. (2004) Identification of estrogenic compounds in wastewater effluent. *Environ Toxicol Chem.* **23,** 2807-2815
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7. Takemura Y. et al.(2007) Metformin suppresses IL-1 β -induced IL-8 production, aromatase activation and proliferation of endometriotic stromal cells. *J CLIN ENDOCRINOL METAB.* **92(8),** 3213-3218
8. Satoh K. et al.(2008) In Vitro screening assay for detecting aromatase activity using rat ovarian microsomes and estrone ELISA. *Biol. Pharm. Bull.* **31(3),** 357-362

Manufactured by Yanaihara Institute Inc.

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