YK170　17 β - Estradiol　EIA

(For measurement of environmental water & culture supernatant)

FOR LABORATORY USE ONLY

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Contents

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

YK170: 17β-Estradiol EIA Kit

（For measurement of environmental water & culture supernatant ）

Ⅰ. Introduction

Estrogen is now known to include over 20 kinds of related steroidal compounds. Among them, estrone (E1), estradiol (E2), and estriol (E3) are most abundant in the human body. These three are highly stable, to which other estrogen - related steroidal compounds are shown to be transformed rapidly in the metabolic processes.

Recently, estrogen is attracting public attention as an environmental pollutant, especially in rivers and wastewater. The assessment of such pollution is therefore an important issue to protect people from the adverse estrogenic effects of the pollutant.

Some immunoassay kits are already available commercially for measurement of estrogen, but only in blood and not in environmental materials and culture supernatant. As a part of the developments of a series of estrogen immunoassay kits, we produced the immunoassay kit for measurement of 17β-estradiol in environmental water and culture supernatant, especially in rivers and wastewater. We present this kit is characterized by its high specificity, sensitivity and easy handling.

YK170　17 β-Estradiol EIA

▼ The assay kit can measure 17β-estradiol in the range of 16.5 - 4000 pg/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 & culture supernatant
Sample volume: 100 μL

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

▼ Precision and reproducibility
Intra-assay CV (%)
Environmental water　4.6-10.0
Culture supernatant　2.7 - 4.4
Inter-assay CV(%)
Environmental water　2.8-13.6
Culture supernatant　5.3 - 11.4

▼ Stability and Storage
Store all of the components at 2-8℃.
24 months from the date of manufacturing.
The expiry date is described 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 17β- estradiol in environmental water and culture supernatant. The kit is characterized for sensitive quantification, high specificity and easy handling.

＜Specificity＞

The EIA kit shows no cross reactivity to testosterone, estrone, progesterone, estriol and cholesterol.

＜Test Principle＞

This EIA kit for determination of 17β-estradiol in environmental water and culture supernatant sample is based on a competitive enzyme immunoassay using combination of highly specific antibody to 17 β-estradiol and biotin-avidin affinity system. The 96-wells plate is coated with goat anti rabbit IgG. Biotinylated 17β-estradiol, 17β-estradiol standard or samples and rabbit anti 17β-estradiol are added to the wells for competitive immunoreaction. After incubation and plate washing, HRP labeled streptavidin (SA-HRP) are added to form HRP labeled streptavidin-biotinylated 17β- estradiol-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by o-phenylenediamine dihydrochloride (OPD) and the concentration of 17β-estradiol is calculated.

Ⅲ. Composition

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	microtiter plate	1 plate (96 wells)	Goat anti rabbit IgG
2.	Standard	lyophilized*	1 vial (400ng)	17β-estradiol
3.	Labeled antigen	lyophilized	1 vial	Biotinylated 17β- estradiol
4.	Specific antibody	lyophilized	1 vial	Rabbit anti 17β- estradiol
5.	SA-HRP solution	liquid	1 bottle (12mL)	HRP labeled streptoavidin
6.	Substrate buffer	liquid	1 bottle (24mL)	0.015% Hydrogen peroxide
7.	OPD tablet	tablet	2 tablets	o-Phenylenediamine dihydrochloride
8.	Stopping solution	liquid	1 bottle (12mL)	1M H_2SO_4
9.	Buffer solution	liquid	1 bottle (25mL)	Phosphate buffer
10.	Washing solution (concentrated)	liquid	1 bottle (50mL)	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. 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 490 nm (or 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 standard (400 ng/vial) with 1mL 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 9.9 mL of buffer solution, that yields 4,000 pg/mL standard solution. The 0.2 mL of the 4,000 pg/mL standard solution is diluted with 0.4 mL of Buffer solution, that yields 1,333.3 pg/mL standard solution. Repeat the same dilution to make each standard of 444.4, 148.1, 49.4, 16.5 pg/mL. Buffer solution is used as 0 pg/mL.

   <Assay range> 16.5-4,000 pg/mL

   If the sample value estimates below the 16.5pg /mL, one more standard solution should be set up. It should be diluted 16.5pg/mL standard solution to 5.5pg/mL in this case, 40 samples can be measured in duplicate. Use the calculated sample value which is between the concentration of 5.5pg/mL～16.5pg/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＞

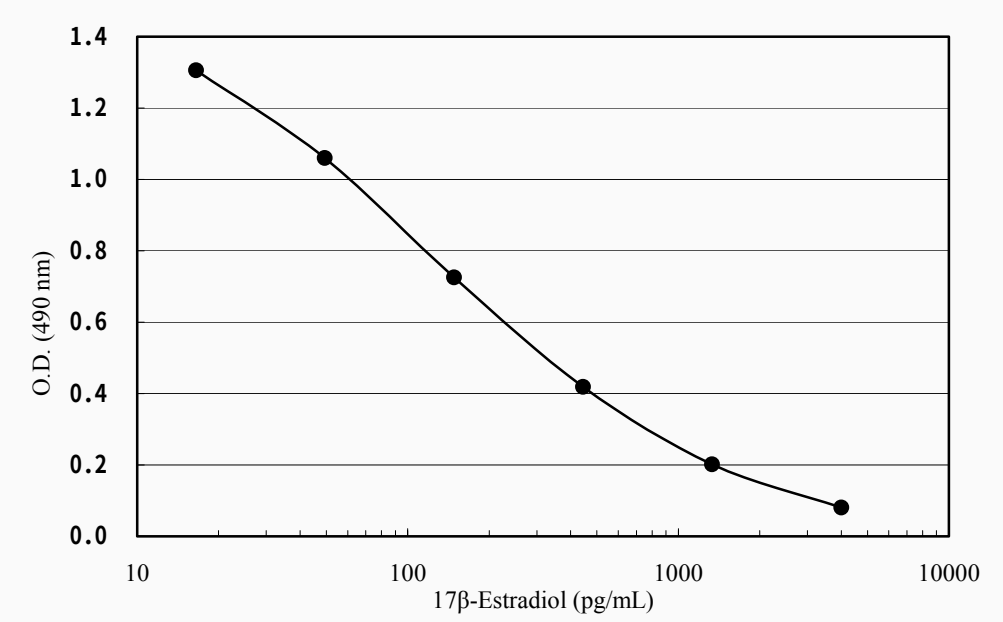
1. Bring all the reagents and samples to room temperature before beginning the test.
2. Add 0.35mL/well of washing solution into the wells and aspirate the washing solution in the wells. Repeat this washing procedure further twice (total 3 times). Finally, invert the plate and tap it onto an absorbent 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, 16.5, 49.4, 148.1, 444.4, 1333.3, 4000 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℃ 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℃) for 2 hour. During the incubation, the plate should be shaker 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 490 nm (or 492nm). 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 semilogarithmic 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 -30℃. Avoid repeated freezing and thawing of samples.
2. 17β-estradiol 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 -30ºC(stable for 1 month).
3. During storage of washing solution (concentrated) at 2-8℃, precipitates may be observed, however they will be dissolved when diluted. Diluted washing solution is stable for 6 months at 2-8℃.
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 4000 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. Perform all the determination in duplicate.
8. Read plate optical absorbance of reaction solution in wells as soon as possible after stopping 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 17β-Estradiol (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
0.0	9.6		
7.8	16.5	17.4	94.8
125.0	147.9	134.6	109.9
2000.0	2572.5	2009.6	128.0

Environmental water B

Added 17β-Estradiol (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
0.0	5.1		
7.8	14.7	12.9	114.0
125.0	132.0	130.1	101.5
2000.0	2669.1	2005.1	133.1

Culture supernatant A (phenol red+)

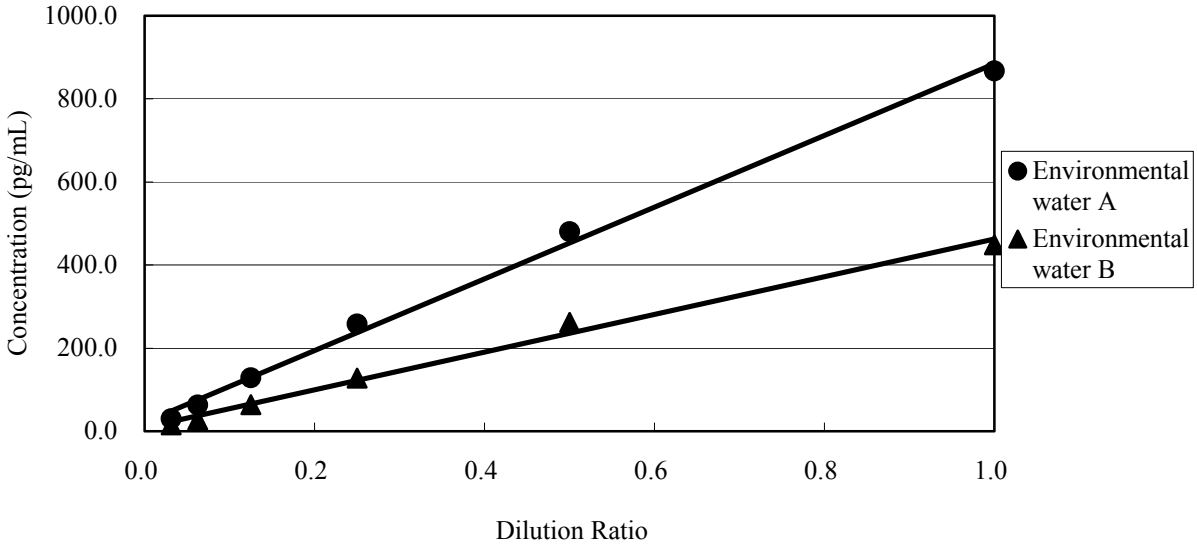
Added 17β-Estradiol (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
0.0	19.7		
20.0	35.1	39.7	88.4
100.0	112.4	119.7	93.9
1000.0	1003.9	1019.7	98.5

Culture supernatant B (phenol red−)

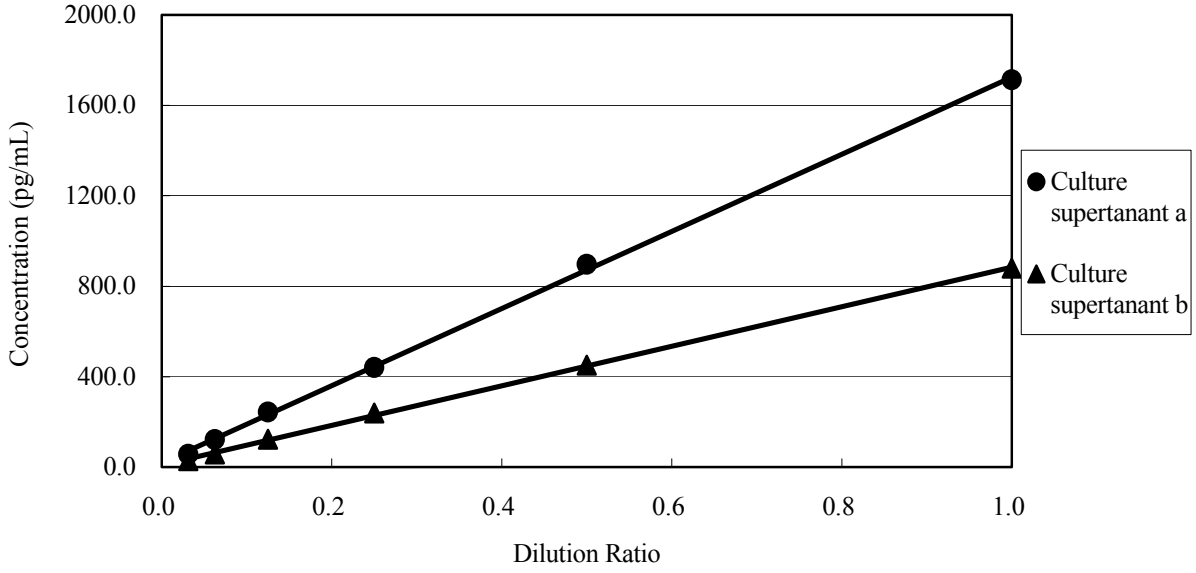
Added 17β-Estradiol (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
0.0	24.6		
20.0	41.2	44.6	92.4
100.0	127.9	124.6	102.6
1000.0	1083.4	1024.6	105.7

< Dilution test >

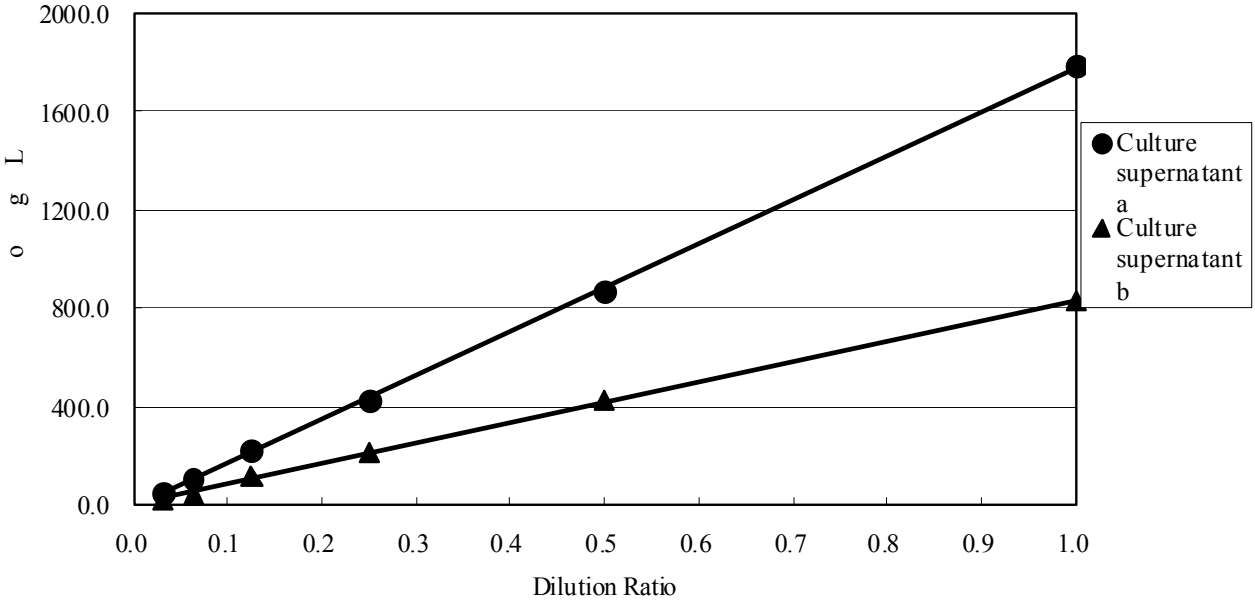
Environmental water



Culture supernatant A (phenol red+)



Culture supernatant B (phenol red−)



<Crossreactivity>

Compounds	Crossreactivity (%)
17β-Estradiol	100
Testosterone	0.28
Estrone	0.94
Progesterone	0.01
d-Aldosterone	0.00
Estriol	0.16
(+)-4-Androsterone-3,17-dione	0.02
Trans-Androsterone	0.02
Mestranol	0.14
Ethynylestradiol	0.03
2-Methoxyestradiol	1.79
Hexestrol	0.00
β-Estradiol 17-(β-D-Glucronide)	0.00
β-Estradiol 3-Glucronide 17-Sulfate	0.02
β-Estradiol 3-(β-D-Glucronide)	37.44
β-Estradiol 3-Sulfate	11.05
β-Estradiol 3-Sulfate 17-Glucronide	0.00
β-Estradiol 3,7-Disulfate	0.00
Cholesterol	0.00

<Precision and reproducibility>

Test sample	Intra-assay CV(%)	Inter-assay CV(%)
Environmental water	4.6-10.0	2.8-13.6
Culture supernatant	2.7-4.4	5.3-11.4

Ⅶ. Stability and Storage

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

< 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. Goda, Y. et al.: Development of the ELISA for detection of hormone-disrupting chemicals. WATER SCIENCE TECHNOLOGY 42(7-8): 81-88, 2000
2. Goda, Y. et al.: Development of the ELISA for detection of estrogenic hormones in environment. IWA 2nd World Water Congress, Berlin, Germany IWA CD-ROM: 269, 2001
3. Nichols, D. J. et al.: Runoff of estrogen hormone 17β-estradiol from poultry litter applied to pasture. JOURNAL OF ENVIRONMENTAL QUALITY 26(4): 1002-1006, 1997

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