Ⅱ. Characteristics

This EIA kit is used for quantitative determination of 17 β- estradiol in environmental water. It has a lot of advantage to perform the assay, such as good quantification and high specificity.

<Specificity>

The EIA kit shows cross reactivity of 100% to 17 β- estradiol and shows no cross reactivity to testosterone, estrone, progesteron and estriol.

<Test Principle>

This EIA kit for determination of 17 β- estradiol in environmental water sample is based on a competitive enzyme immunoassay using combination with highly specific antibody to 17 β- estradiol and biotin – avidin affinity system. The 96 wells plate is coated with goat anti rabbit IgG. Biotinylated 17 β- estradiol, 17 β- estradiol standard or samples and rabbit anti 17 β- estradiol are added to the wells for competitive immunoreaction. After rinsing out excess 17 β- estradiol, HRP labeled streptoavidins are added to bind to the antigen – antibody complex so that HRP labeled streptoavidins — biotinylated 17 β- estradiol — antibody complexs are formed on the surface on the wells. Finally, excess HRP labeled streptoavidins are rinsed out and HRP enzyme activity is determined and the concentration of 17 β- estradiol 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	1 vial (400ng)	17 β-estradiol
3. Labeled antigen	lyophilized	1 vial	Biotinylated 17 β-estradiol
4. Specific antibody	lyophilized	1 vial	Rabbit anti 17 β-estradiol
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 hydrochloride
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. . . . . Microtitration plate

IV. Method

<Equipment required>

1. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
2. Photometer for microtitration 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 microtitration plate and dispenser for approximately 0.35 mL 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 (400ng/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.
2. Preparation of labeled antigen : Reconstitute labeled antigen with 6 mL of distilled or deionized water .
3. Preparation of specific antibody : Reconstitute labeled antigen 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＞

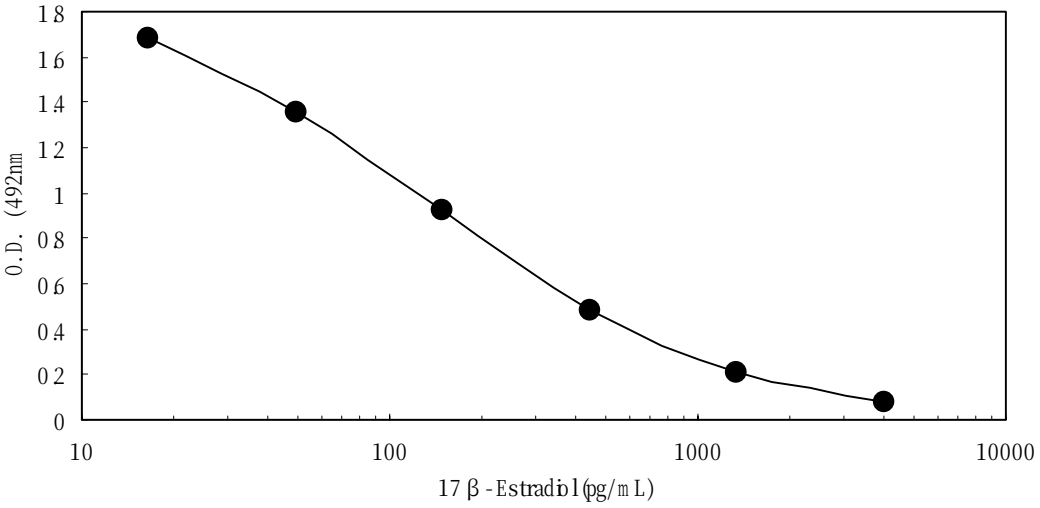
1. Warm up the reagents and samples to room temperature before beginning the test.
2. Wash the wells three times with approximately 0.35 mL/well of washing solution.
3. Fill 50 μL of labeled antigen and add 100 μL of each of standard solutions (0, 16.5, 49.4, 148.1, 444.4, 1333.3, 4000 pg/mL) or samples, then introduce 50 μL of specific antibody into the wells.
4. Cover the plate with adhesive foil and incubate it at 4℃ overnight (17～19hours).
5. Warm up the plate to room temperature (1hour) and take off the adhesive foil, aspirate and wash the wells 4 times with approximately 0.35 mL/well of 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 rotated with a plate rotator.
8. Take off the adhesive foil, aspirate and wash the wells 4 times with approximately 0.35 mL/well of washing solution.
9. Add 100 μL of substrate solution into the wells, cover the plate with adhesive foil and incubate it for 20 minutes at room temperature.
10. Add 100 μL of stopping solution into the wells to stop reaction.
11. Read the optical absorbance of the wells at 492nm..
12. 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 17 β- estradiol 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 to be tested at a later time, they should be divided into test tubes in small amount and frozen at or below – 30℃. Avoid repeated freezing and thawing of samples.
2. 17 β– estradiol standard, Labeled antigen, Specific antibody and OPD solution should be prepared immediately before use in assay using clean test tubes or vessels. Diluted washing solution is stable for 6 months at 2 to 8℃.
3. During storage of washing solution (concentrated) at 2 to 8℃, precipitates may be observed, however they will be dissolved when diluted.
4. As pipetting operations may affect with the precision of the assay, pipette precisely standard solutions or samples into each well of plate. And use 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 within the assay range.
6. During incubation with SA–HRP solution at room temperature, the test plate should be rotated gently by plate rotator to promote immunoreaction.
7. During continuous rotation of test plate, the plate rotator may be heated up. It is recommended to place styrene form or plywood between the plate and the rotator.
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 test>

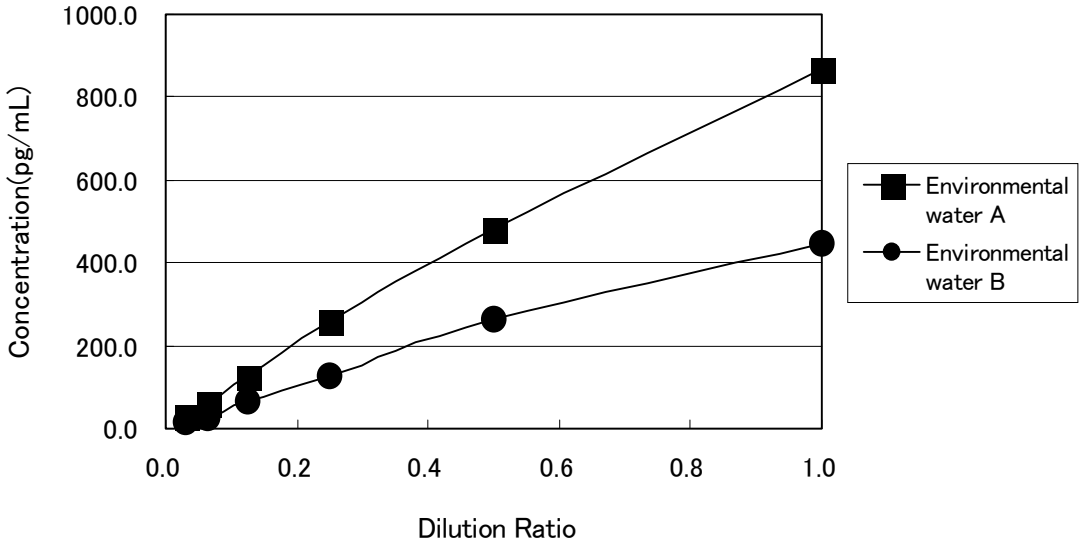
<Environmental water A>

Added 17 β-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 17 β-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

<Dilution test>



<Crossreactivity>

Compounds	Crossreactivity (%)
17 β-Estradiol	100
Testosterone	0.28
Estrone	0.94
Progesteron	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

<Precision and reproducibility test>

Intra-assay		Inter-assay	
Environmental water CV(%)	4.6 10.0	Environmetal water CV(%)	2.8 13.6

Ⅶ. Stability and Storage

<Storage>
Store all of the components at 2 to 8℃, and protect from light

<Shelf life>
6 month from the date of manufacturing
(The expiry date is described on the label of kit.)

<Package>
For 96 tests per 1 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