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ABRAXIS

Ecologiena

17β-Estradiol(E2) ELISA KIT

(Microplate)

User's Guide

17β-Estradiol ELISA KIT (Microplate) - TABLE OF CONTENTS

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The design of the product is under constant review and every effort is made to keep this guide up to date, the right is reserved to change specifications and equipment at any time without prior notice.

Measuring Principle (Competitive ELISA)

1. Competitive Reaction

The test is based on the recognition of 17β-Estradiol by specific monoclonal antibodies. 17β-Estradiol present in the sample and an 17β-Estradiol-enzyme conjugate (i.e. 17β-Estradiol labeled with a coloring enzyme) are premixed and added into each well of a microplate, and allowed to compete for limited number of binding sites of specific antibodies immobilized on the surface of the wells. When the 17β-Estradiol concentration is higher relative to the enzyme conjugate, the 17β-Estradiol will predominantly bind the antibody and vice versa.

2. Chromogenic Reaction

Unbound 17β-Estradiol and excess antigen-enzyme conjugates are washed out. The chromogenic substrate is then added to develop color in conjunction with the enzyme conjugate. The amount of 17β-Estradiol-enzyme conjugate remaining bound to the antibody will determine the color intensity. The higher 17β-Estradiol concentration in sample, for example, leads to less antigen-enzyme conjugate bound to the antibody binding sites in a microplate well, generating a lighter color, i.e. lower absorbance.

3. Quantitative Analysis

The standard curve, a dose-response curve obtained from known concentrations of 17β-Estradiol standards, is determined from the absorbance at 450nm. The 17β-Estradiol concentration in each sample is accurately calculated by interpolation using the absorbance intensity obtained from the standard curve.

Kit Content

	Contents	Volume	Quantity	Storage
1	MoAb-Coated Microplate	96 Wells	1 Plate	2-8°C
2	17β-Estradiol Standards (Ready to use in 10%MeOH). Concentrations: 0, 0.05, 0.15, 0.4, 1.0 ug/L)	1.5 mL	1 Vial each	2-8°C
3	Antigen-enzyme Conjugate	7mL	2 Vials	2-8°C
4	Buffer Solution– *white cap*	8mL	2 Vials	2-8°C
5	Uncoated Microplate	96 Wells	1 Plate	---
6	Wash Solution (6-fold concentration)	50mL	1 Vial	2-8°C
7	Color Solution	15 mL	1 Vial	2-8°C
9	Stop Solution– *black cap*	15mL	1 Vial	2-8°C
10	Instruction Booklet	---	1	---

Other Essential or Recommended Reagents/Materials

NOTE: For reagents/materials necessary for sample concentration (water), serum extraction or any other application, please refer to application bulletins.

Essential

1. Disposable test tubes (e.g. IWAKI, item No. 9831-1207)
2. Glass fiber filters (e.g. ADVANTEC Co., item No. 36481047 ϕ47mm) and filtering equipment
3. Micropipettes (10µL -100µL and 100µL -1000µL) and tips

4. Multichannel pipettes (50µL -300µL) and tips
5. Microplate reader (450nm wavelength) (e.g. TECAN, SPECTRA Classic)
6. Stop watch
7. Methanol (for HPLC grade)

Recommended

8. Plate cover
9. Strip ejector (e.g. COSTAR, No.2578)

IMPORTANT

- Comparative tests should be needed if an alternate supplier is used for specified reagents or materials.

Test Protocol

IMPORTANT

- For research use only, not for human use and/or diagnostic use.
- Take out all the kit contents from the refrigerator and let them reach room temperature (18-25°C) prior to the assay.
- Do not mix reagents from different kits.
- Store reagents under storage conditions defined in this guide.
- Do not use expired kits.
- Dispose of kit components after use in accordance with applicable regulations.
- Prepare more than one sample from each location or standard dilution for more accurate quantification.

CAUTION

Wear appropriate protective clothing, gloves and eyewear to avoid any accidental contacts.

1. Sample Filtration

Filter raw water samples through the specified glass fiber filter (1µm pore diameter). If no sample concentration is needed, dilute samples (in glass tubes) with methanol to obtain a final concentration of 10% methanol (i.e. 900 uL of sample and 100 uL of methanol). If a lower assay sensitivity is needed then follow directions on sample concentration-pre treatment procedure. For samples other than water please follow appropriate technical bulletins.

2. Antigen-enzyme Conjugate Solution

Reconstitute antigen-enzyme conjugate powder with 7mL of buffer solution to prepare antigen-enzyme conjugate solution.

- Store the conjugate solution at 2-8°C; it will be stable for approximately 2 weeks.
- Mix by filling the tip and expelling the contents with a pipette.
- Mix a pair of reconstituted solutions when you use them altogether.

3. Mixture of Standard/Sample and Conjugate Solution

Transfer 100µL of E2 standard (or sample), pretreated and adjusted to contain 10% methanol, and 100µL of conjugate solution into each well of the uncoated microplate and mix by filling the tip and expelling the contents with a pipette.

- Dispense standard solution first, and then add conjugate solution to avoid non-specific adsorption on the inner surface of the well.
- Use 10% methanol solution as a blank.

4. Competitive Reaction

Dispense 100μL aliquots of the above mixture into each coated well of the microplate. Incubate the microplate for 60 minutes at room temperature (18-25°C).

- Split the microplate, with a strip ejector for example, to use necessary number of wells. This microplate is breakable into 12 strips, each of which consists of 8 wells. Place back the unused plate strips in the packet, seal and store them at 2-8°C.
- Place the pipette tip on the well bottom and slowly transfer the content so that any mixture solution does not touch or remain on the non-coated area of the well.
- Cover a microplate with film to avoid contamination.
- Do not move or shake a microplate during the reaction.
- Be sure not to generate bubbles when you transfer liquid to avoid erroneous reading. To remove them, tap a plate lightly.
- Secure the constant reaction time for each well, particularly to measure many samples.

5. Wash Solution

Dilute 6-fold wash solution concentrate with distilled water at a ratio of 1:5 to prepare a wash solution. Rinse each well with approximately 300 μL of the solution and repeat 3 times. Be sure that all the wash solution is removed from the microplate prior to the assay.

- The wash solution must be stored at 2-8°C; it will be stable approximately for a month after preparation.
- Be sure that all wash solution is removed from the microplate prior to the assay.
- Keep the wash solution and dispose in accordance with local regulations.

6. Chromogenic Reaction

Dispense 100μL of the Color Solution into each microplate well and incubate the microplate for 30 minutes at room temperature (18-25°C). Then, add 100μL of stop solution (with a black cap) to stop the reaction.

- Make sure that there is a constant reaction time for each well, particularly when analyzing many samples.

7. Quantification

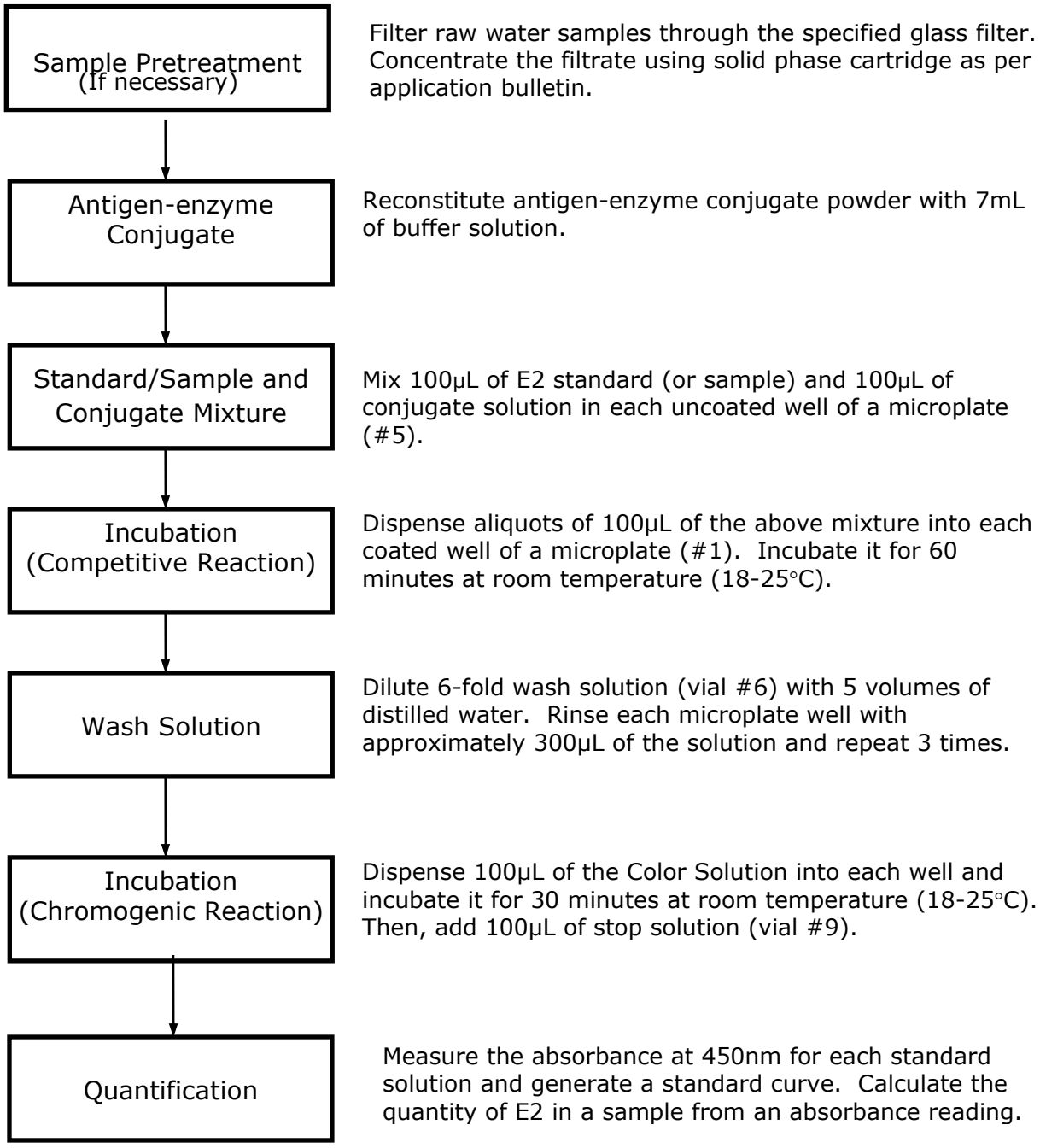
Measure the absorbance at 450nm for each standard solution and generate a standard curve. Calculate the quantity of E2 in a sample from an absorbance reading.

- Measure the absorbance within 15 minutes after the reaction is stopped.
- Prepare a standard curve based on at least duplicate standards for every assay.
- The assay must be performed within the range between 0.05 μg/L and 1.0 μg/L. Samples of concentration beyond 1 μg/L must be diluted with 10% methanol and re-tested.

APPENDIX

1. Flowchart for 17β-Estradiol Measurement

Note: Take out all the kit contents from a refrigerator and let them reach room temperature (18-25°C) prior to the assay.
<Please follow the steps describing in the text: Test Protocol>



2. Cross-reactivity Pattern

Estrogens

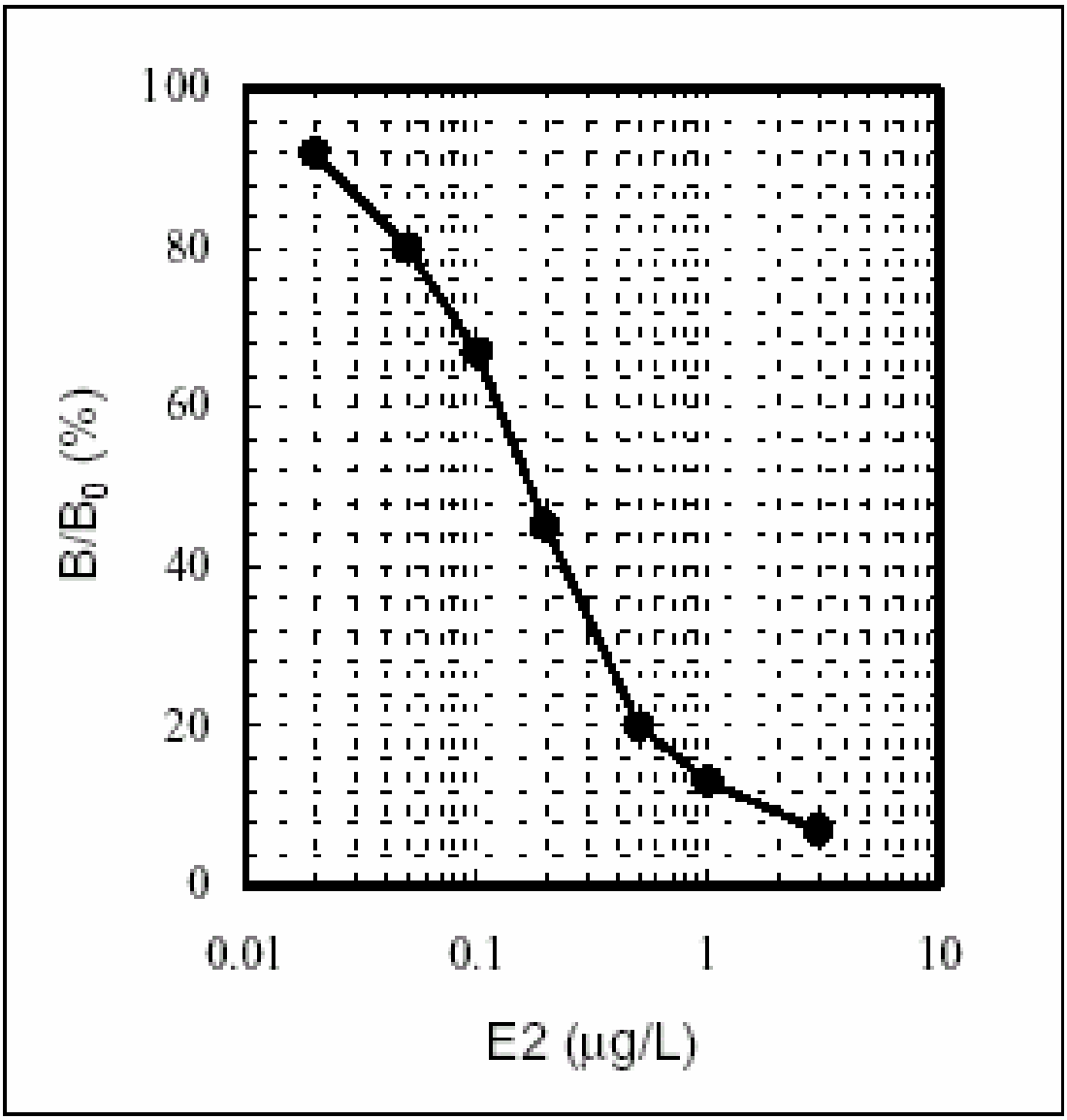
Compound	%reactivity
Estron (E1)	1.3
2-methoxy E1	<0.4
E1-3-sulfate	1.0
17β-Estradiol (E2)	100.0
16-keto E2	16.0
2-methoxy E2	2.0
E2-17-glucronide	<0.4
E2-3-glucronide	16.0
E2-3-sulfate-17-	<0.4
Estriol (E3)	0.6
16-epi-E3	0.5
E3-16-glucronide	<0.4

Other Hormons

Compound	%reactivity
cis-Androsterone	<0.03
trans-Androsterone	<0.03
Cholesterol	0.46
Dehydroisoandrosterone	<0.03
5α-Dihydrotestosterone	0.38
Hydrocortisone	0.38
Pregnenolone	0.35
Testosterone	<0.03

3. 17β-Estradiol Standard Curve

Samples containing 17β-Estradiol within the dynamic range (0.05 µg/L and 1.0 µg/L can be directly applied to the assay only after filtration. Samples outside of the range must be either diluted with 10% methanol or concentrated with solid phase extraction prior to analysis. Coefficient of variation (CV) are generally under 10% throughout the dynamic range.



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