Version 23-04/24

User's Manual

Total Estriol ELISA Kit

REF **DEIA1843**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

Competitive immunoenzymatic colorimetric method for determination of Total Estriol concentration in human serum or plasma.

Principles of Testing

Total Estriol (antigen) in the sample competes with horseradish-peroxidase Estriol (enzyme-labelled-antigen) for binding onto the limited number of anti Estriol (antibody) sites on the microplates (solid phase). After incubation, the bound/total separation is performed by a simple solid-phase washing. The enzyme substrate (H_2O_2) and the TMB-Sustrate (TMB) are added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbances are determined. Total Estriol concentration in the sample is calculated based on a series of standard. The colour intensity is inversely proportional to the Total Estriol concentration in the sample.

Reagents And Materials Provided

1. Total Estriol Standards S1 – S4, 4× (1 vial = 1 mL)
2. Incubation Buffer (1 bottle) 30 mL; Phosphate buffer 50 mM pH 7.5; BSA 1 g/L, stabiliser
3. Enzyme Conjugate (1 bottle) 0.4 mL, Estriol-HRP conjugate
4. Coated Microplate (1 microplate breakable), Anti-Estriol IgG adsorbed on microplate
5. TMB Substrate Solution (1 bottle) 12 mL, H_2O_2-TMB 0.26 g/L, (avoid any skin contact)
6. Stop Solution (1 bottle) 12 mL; Sulphuric acid 0.15 mol/L, (avoid any skin contact)

Note: Open the bag of the Coated Microplate only when it is at room temperature and close immediately after use. Do not remove the adhesive sheet from the unused strips.

Materials Required But Not Supplied

1. Distilled water.
2. Automatic dispenser
3. Microplates reader

Storage

Store all reagents between 2-8°C in the dark.

Specimen Collection And Preparation

The determination of Total Estriol should be performed in human serum or plasma. Store samples at -20°C if the determination is not performed on the same day of sample collection.

Reagent Preparation

Preparation of the Standard (S1,S2,S3,S4)

Before use, mix for 2 min. with rotating mixer. The standards have the following concentration of Estriol: S1 2.0ng/mL, S2 20.0ng/mL, S3 80.0ng/mL,S4 200.0ng/mL.

Note: Stable until the expiration date of the kit. Once open stable for six months at +4°C.

Preparation of diluted Conjugate

Prepare immediately before use. Add 10 µL Conjugate (reagent 3) to 2.0 mL of Incubation Buffer (reagent 2).

Mix gently for 5 minutes, with rotating mixer. Stable for 3 hours at room temperature (22-28°C).

Assay Procedure

As it is necessary to perform the determination in duplicate, prepare two wells for each of the four points of the standard curve (S1-S4), two for B0 and for each sample, one for Blank.

Pipette:

	B_0	Standard	Sample	Blank
Incubation Buffer	20 µL	---	---	---
Sample	---	---	20 µL	---
Standards S_1 -S_4	---	20 µL	---	---
Diluted Conjugate	200 µL	200 µL	200 µL	---

Incubate at 37°C for 1 hour. Remove the contents from each well; wash the wells with 300 µL of distilled water. Repeat the washing procedure again for a total of two washing steps by draining the water completely.

Pipette:

	B_0	Standard	Sample	Blank
TMB Substrate	100 µL	100 µL	100 µL	100 µL

Incubate at 22-28°C for 15 minutes in the dark.

Pipette:

	B_0	Standard	Sample	Blank
Stop solution	100 µL	100 µL	100 µL	100 µL

Read the absorbance (E) at 450 nm against Blank.

Calculation

1. Calculate the mean of the absorbance (Em) for each point of the standard curve and of each sample.
2. Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.

Typical Standard Curve

Plot the values of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points.

Precautions

1. The reagent contain Proclin 300 as preservative.
2. Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants.
3. Maximum precision is required for reconstitution and dispensation of the reagents.
4. Do not use different lots of reagents.
5. Do not use heavily hemolized samples.
6. This method allows the determination of Total Estriol from 2 ng/mL to 200.0 ng/mL.

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