CE

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DCM022-11
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ESTRADIOL SALIVA ELISA

for routine analysis

Direct immunoenzymatic determination of Estradiol in saliva.

LOT
See external label

2°C 8°C

Σ = 96 tests

REF DKO022

INTENDED USE

The Eagle Biosciences Estradiol Saliva ELISA Assay is a competitive immunoenzymatic colorimetric method for quantitative determination of Estradiol concentration in saliva. The Estradiol Saliva ELISA Assay kit is intended for research use only and is not to be used for diagnostic procedures.

1. CLINICAL SIGNIFICANCE

Estradiol (17β-estradiol) is a sex hormone. It represents the major estrogen in humans. Estradiol has not only a critical impact on reproductive and sexual functioning, but also affects other organs including bone structure.

During the reproductive years most estradiol in women is produced by the ovaries, smaller amounts of estradiol are also produced by the adrenal cortex. In men, the testes produce estradiol.

During pregnancy estrogen levels including estradiol rise steadily towards term. Estradiol increases due to placental production.

In adult premenopausal women, ovarian estradiol production is stimulated by the interactions of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) throughout the menstrual cycle.

In adult women, estradiol levels are measured in the evaluation of fertility and menstrual irregularities, and to monitor ovarian follicular function during induction of ovulation

In the female, estradiol acts as a growth hormone for tissue of the reproductive organs.

The development of secondary sexual characteristics in women is driven by estradiol. Estradiol is involved in man feritility.

Estradiol regulate the bone maintenance. Women who past the menopause experience an accelerated loss of bone mass due to a relative estrogen deficiency.

Estradiol affects the production of multiple proteins including lipoproteins, binding proteins, and proteins responsible for blood clotting.

Estrogens have been found to have neuroprotective function.

Estrogen is considered an oncogene as its supports certain cancers, notably breast cancer and cancer of the uterine lining. In addition there are several benign gynecologic conditions that are dependent on estrogen such as endometriosis, leiomyomata uteri, and uterine bleeding.

2. PRINCIPLE

The Estradiol (antigen) in the sample competes with the antigenic Estradiol conjugated with horseradish peroxidase (HRP) for binding to the limited number of antibodies anti Estradiol coated on the microplate (solid phase).

After incubation, the bound/free separation is performed by a simple solid-phase washing.

Then, the enzyme HRP in the bound-fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blu color that changes into yellow when the Stop Solution (H_2SO_4) is added.

The colour intensity is inversely proportional to the Estradiol concentration of in the sample.

Estradiol concentration in the sample is calculated through a calibration curve.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. Calibrators (5 vial, 1 mL each)

CAL0	REF **DCE002/2206-0**
CAL1	REF **DCE002/2207-0**
CAL2	REF **DCE002/2208-0**
CAL3	REF **DCE002/2209-0**
CAL4	REF **DCE002/2210-0**

2. Controls (2 vials, 1 mL each)

Control A	REF **DCE045/2203A-0**
Control B	REF **DCE045/2203B-0**

Controls Concentration is indicated on the Certificate of Analysis

3. Incubation Buffer (1 vial, 30 mL)
HEPES buffer pH 7.5, BSA 1 g/L
REF **DCE001/2201-0**

4. Conjugate (1 vial, 1 mL)
Estradiol conjugated with Horseradish peroxidase(HRP)
REF **DCE002/2202-0**

5. Coated Microplate (1 breakable microplate)
Anti Estradiol antibody adsorbed on microplate
REF **DCE002/2203-0**

6. TMB Substrate (1 vial, 15 mL)
H_2O_2-TMB 0.26 g/L *(avoid any skin contact)*
REF **DCE004-0**

7. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15 mol/L *(avoid any skin contact)*
REF DCE005-0

7. 50X Conc. Wash Solution (1 vial, 20 mL)
NaCl 45 g/L; Tween-20 55 g/L **REF DCE006-0**

3.2. Reagents necessary not supplied
Distilled water.

3.3. Auxiliary materials and instrumentation
Automatic dispenser.
Microplates reader (450 nm, 620-630 nm)
Saliva Collection Device **REF DKO063**
Salivette Sarstedt **REF 51.1534.500**

Note

Store all reagents at 2÷8°C in the dark.
Open the bag of reagent 5 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, the microplate is stable until the expiry date of the kit.
Do not remove the adhesive sheets on the unused strips

4. WARNINGS
- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Some reagents contain small amounts of Proclin 300^R as preservative. Avoid the contact with skin or mucosa.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.
- This method allows the determination of Estradiol from 1 pg/mL to 100 pg/mL.
- The clinical significance of the determination Estradiol can be invalidated if the patient was treated with cortisone or natural or syntetic steroids.

5. PRECAUTIONS
- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of reagents.
- Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of the Calibrators (C_0…C_4)
Before use, mix for 5 minutes with a rotating mixer.
The Calibrators are ready to use and have the following concentration of Estradiol:

	C_0	C_1	C_2	C_3	C_4
pg/mL	0	1	5	20	100

Once opened, the Calibrators are stable at 2-8°C for 6 months.
For SI UNITS: ng/mL x 3,76 = nmol/L

6.2. Preparation of Wash Solution
Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled water to a final volume of 1000 mL prior to use. For smaller volumes respect the 1:50 dilution ratio. The diluted wash solution is stable for 30 days at 2÷8°C.

6.3. Preparation of Diluted Conjugate
Prepare immediately before use.
Add 10 µL of Conjugate (reagent 3) to 1.0 mL of Incubation Buffer (reagent 2). Mix gently. Stable 3 hours at room temperature (22÷28°C).

6.4. Preparation of Samples and Controls

The determination of Estradiol with this kit should be performed in saliva samples.
It is recommended to collect saliva samples with a centrifuge glass tube and a plastic straw, with the Diametra *Saliva Collection Device* or with the "Salivette" (Sarstedt, Ref. 511534500). Other commercially available sample collector devices have not been tested.
The controls are ready to use.

6.4.1. Method and Limitations

Collect saliva samples at the times indicated.
If no specific instructions have been given, saliva samples may be collected at any time; however the following should be noted:

a) If saliva collection is carried out in the morning ensure that this is carried out prior to brushing teeth
b) During the day allow 1 hour after a meal, oral intake of pharmaceutical drugs or tooth cleaning.
c) It is very important that a good clear sample is received – i.e. no contamination with food, lipstick, blood (bleeding gums) or other extraneous materials.

6.4.2. Saliva Processing Instructions with *Saliva Collection Device Diametra*

1) Let the saliva flow down trought the straw into the centrifuge glass tube.
2) Centrifuge the sample for 15 minutes at 3000 rpm
3) Store at – 20°C for at least 1 hour
4) Centrifuge again for 15 minutes at 3000 rpm
5) The saliva sample is now ready to be tested.
6) Store the sample at 2÷8°C for one week or at – 20°C for longer time.

6.4.3. Saliva Processing Instructions with *Salivette Sardstedt*

1) Remove the swab from the suspended insert of the Salivette
2) Gently chewing the swab for 1 minute produces a sufficient quantity of saliva.
3) Replace the swab into the Salivette and firmly close the tube using the stopper.
4) Centrifuge the Salivette for 2 minutes at 1000g (rcf) for saliva generation.
5) Remove the insert complete with the swab from the centrifuge vessel and discard. The clear saliva is now ready for analysis (at least 1 mL of saliva should be recovered with this method).

6.5. Procedure

- **Allow all reagents to reach room temperature (22-28°C).** At the end of the assay, store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_4), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample / Control	Blank
Calibrator C_0-C_4	**100 µL**		
Sample / Control		**100 µL**	
Diluted Conjugate	**100 µL**	**100 µL**	
Incubate at +37°C for 2 hours. Remove the contents from each well. Wash the wells 3 times with 300 µL of diluted wash solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate at room temperature (22÷28°C) for 15 minutes in the dark.			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake gently the microplate. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

7. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of Estradiol for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the calibration curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8. RESULTS

8.1. Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the calibration curve (C_0-C_4) and of each sample.

8.2. Calibration curve

Plot the mean value of absorbance (Em) of the Calibrators (C_0-C_4) against concentration. Draw the best-fit curve through the plotted points. (es: Four Parameter Logistic).

8.3. Calculation of Results

Interpolate the values of the samples on the calibration curve to obtain the corresponding values of the concentrations expressed in pg/mL.

9. REFERENCE VALUES

As the values of salivary Estradiol have a cicardian pattern we suggest to collect the samples at the same hour (8 A.M.):
The following values can be used as preliminary guideline until each laboratory established its own normal range.

		pg/mL
WOMEN:	Follicular phase	1 – 20
	Ovulatory peak	10 – 40
	Luteinic phase	5 – 25
	Menopause	< 10
CHILDREN:		< 20
MEN:		< 20

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

10. PERFORMANCE AND CHARACTERISTICS

10.1. Precision

10.1.1. Intra Assay Variation

Within run variation was determined by replicate the measurements (20x) of two different control samples in one assay. The within assay variability is ≤ 10.3%.

10.1.2. Inter Assay Variation

Between run variation was determined by replicate the measurements (10x) of three different control samples in different lots of kit. The between assay variability is ≤ 13.6%

10.2. Accuracy

The recovery of 2.5 – 10 – 50 pg/mL of Estradiol added to "saliva-free" sample gave an average value (±SD) of 106.84% ± 7.80% with reference to the original concentrations.

10.3. Sensitivity

The lowest detectable concentration of Estradiol that can be distinguished from the Calibrator 0 is 0.5 pg/mL at the 95% confidence limit.

10.4. Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

Estradiol	100%
Estrone	2%
Estriol	0.39%
Testosterone	0.02%
Cortisol	$< 7x10^{-3}$%
Progesterone	$< 3x10^{-4}$%
Dhea-s	$< 1x10^{-4}$%

10.5. Correlation

Diametra Estradiol saliva ELISA kit was compared to another commercially available Estradiol saliva assay. 22 saliva samples were analysed according in both test systems.
The linear regression curve was calculated:
(Diametra kit) = 0.98*(DRG Elisa kit) + 0.08
$r^2 = 0.961$

11. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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	DIA.METRA SRL	
Mod. PIS	**PACKAGING INFORMATION SHEET**	

IT *Spiegazione dei simboli* **GB** *Explanation of symbols* **FR** *Explication des symboles* **ES** *Significado de los simbolos*
DE *Verwendete Symbole* **PT** *Explicaçao dos simbolos*

IVD	DE ES FR GB IT PT	In vitro Diagnostikum Producto sanitario para diagnóstico In vitro Dispositif medical de diagnostic in vitro In vitro Diagnostic Medical Device Dispositivo medico-diagnostico in vitro Dispositivos medicos de diagnostico in vitro		DE ES FR GB IT PT	Hergestellt von Elaborado por Fabriqué par Manufacturer Produttore Produzido por
REF	DE ES FR GB IT PT	Bestellnummer Nûmero de catálogo Réferéncès du catalogue Catalogue number Numero di Catalogo Número do catálogo	yyyy-mm	DE ES FR GB IT PT	Herstellungs datum Fecha de fabricacion Date de fabrication Date of manufacture Data di produzione Data de produção
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	DE ES FR GB IT PT	Gebrauchsanweisung beachten Consultar las instrucciones Consulter le mode d'emploi Consult instructions for use Consultare le istruzioni per l'uso Consultar instruções para uso	LOT	DE ES FR GB IT PT	Chargenbezeichnung Codigo de lote Numero de lot Batch code Codice del lotto Codigo do lote
Σ = xx	DE ES FR GB IT PT	Ausreichend für "n" Tests Contenido suficiente para "n" tests Contenu suffisant pour "n" tests Contains sufficient for "n" tests Contenuto sufficiente per "n" saggi Contém o suficiente para "n" testes	**Cont.**	DE ES FR GB IT PT	Inhalt Contenido del estuche Contenu du coffret Contents of kit Contenuto del kit Conteúdo do kit
Max Min	DE ES FR GB IT PT	Temperaturbereich Límitaciôn de temperatura Limites de température de conservation Temperature limitation Limiti di temperatura Temperaturas limites de conservação			

SUGGERIMENTI PER LA RISOLUZIONE DEI PROBLEMI/TROUBLESHOOTING

ERRORE CAUSE POSSIBILI/ SUGGERIMENTI

Nessuna reazione colorimetrica del saggio

- mancata dispensazione del coniugato
- contaminazione del coniugato e/o del Substrato
- errori nell'esecuzione del saggio (es. Dispensazione accidentale dei reagenti in sequenza errata o provenienti da flaconi sbagliati, etc.)

Reazione troppo blanda (OD troppo basse)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo breve, temperatura di incubazione troppa bassa

Reazione troppo intensa (OD troppo alte)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo lungo, temperatura di incubazione troppa alta
- qualità scadente dell'acqua usata per la soluzione di lavaggio (basso grado di deionizzazione)
- lavaggi insufficienti (coniugato non completamente rimosso)

Valori inspiegabilmente fuori scala

- contaminazione di pipette, puntali o contenitori- lavaggi insufficienti (coniugato non completamente rimosso)

CV% intrasaggio elevato

- reagenti e/o strip non portate a temperature ambiente prima dell'uso
- il lavatore per micropiastre non lava correttamente (suggerimento: pulire la testa del lavatore)

CV% intersaggio elevato

- condizioni di incubazione non costanti (tempo o temperatura)
- controlli e campioni non dispensati allo stesso tempo (con gli stessi intervalli) (controllare la sequenza di dispensazione)
- variabilità intrinseca degli operatori

ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers

insufficient washing (conjugates not properly removed) too high within-run

- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run - incubation conditions not constant (time, CV % temperature)

- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation