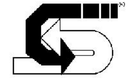

# DRG® Salivary Free Estriol ELISA (SLV-3653)

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**This kit is intended for Research Use Only.**

**Not for use in diagnostic procedures.**

**Intended Use**

The **DRG Salivary Free Estriol ELISA** is an enzyme immunoassay for measurement of free estriol in saliva.

## 1 PRINCIPLE OF THE TEST

The DRG Salivary Free Estriol ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.

The microtiter wells are coated with an anti-Estriol IgG antibody. Endogenous unconjugated ("free") Estriol of a sample competes with an Estriol-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase conjugate is inversely proportional to the concentration of Estriol in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of free Estriol in the sample.

## 2 PRECAUTIONS

1. This kit is for research use only. For professional use only.
2. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
3. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
4. Avoid contact with *Stop Solution* containing 0.15 M $H_2SO_4$. It may cause skin irritation and burns.
5. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
6. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
7. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
8. Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
9. Do not use reagents beyond expiry date as shown on the kit labels.
10. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
11. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.

12. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
13. Safety Data Sheets for this product are available upon request directly from DRG Instruments GmbH. The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

## 3 REAGENTS

### 3.1 Reagents provided

1. ***Microtiterwells***, 12 x 8 (break apart) strips, 96 wells;
   Wells coated with a anti-Estriol IgG antibody.
2. ***Standard (Standard 0-5)*****,** 6 vials, 1 mL, ready to use;
   Concentrations: 0 – 2.5 – 15 – 100 – 600 – 4000 pg/mL.
   Conversion: pg/mL x 3.5 = pmol/L
3. ***Control*** L & M, 2 vials, 1.0 mL, ready to use;
   For control values and ranges please refer to vial label or QC-Datasheet.
4. ***Enzyme Conjugate concentrate***, 1 vial,1 mL,
   Estriol conjugated to horseradish peroxidase,
   see „Preparation of Reagents".
5. ***Incubation Buffer***, 1 vial, 30 mL, ready to use.
   Phosphate buffer pH 7.4, BSA 1g/L
6. ***Substrate Solution***, 1 vial, 15 mL, ready to use;
   Tetramethylbenzidine (TMB).
7. ***Stop Solution***, 1 vial, 15 mL, ready to use;
   contains 0.15 mol/L $H_2SO_4$.
   Avoid contact with the stop solution. It may cause skin irritations and burns.
8. ***Wash Solution***, 1 vial, 20 mL (50X concentrated);
   contains Phosphate buffer 50 mM pH 7.4; Tween20 1gr/l
   see „Preparation of Reagents".

### 3.2 Equipment and material required but not provided

– A microtiter plate calibrated reader (450 ± 10 nm), (e.g. the DRG Instruments Microtiter Plate Reader).
– Calibrated variable precision micropipettes.
– Absorbent paper.
– Distilled or deionized water
– Timer
– Saliva Collection Device
– Semi logarithmic graph paper or software for data reduction

### 3.3 Storage and stability of the Kit

When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again.

### 3.4 Preparation of Reagents

Allow all reagents and required number of strips to reach room temperature prior to use.

***Standards***
Before use, mix the standards for 5 minutes with a rotating shaker.

***Wash Solution***
Add deionized water to the 50X concentrated Wash Solution.
Dilute concentrated *Wash Solution* (20 mL) with distilled or deionized water to a final volume of 1000 mL.
*Store at room temperature until expiration date printed on label of the concentrate vial.*

***Enzyme Conjugate***
Add 10 µl Enzyme Conjugate concentrate to 1.0 mL of Incubation Buffer. Mix gently.
*Stability of the prepared Enzyme-Conjugate : Stable for 3 hours at 22°C - 28°C*
**Prepare immediately before use.**

### 3.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

### 3.6 Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

## 4 SPECIMEN COLLECTION AND PREPARATION

Eating, drinking, chewing gums or brushing teeth should be avoided for 30 minutes before sampling. Otherwise, it is recommended to rinse mouth thoroughly with cold water 5 minutes prior to sampling.

Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).

If there is visible blood contamination the specimen, it should be discarded, rinse the sampling device with water, wait for 10 minutes and take a new sample.

*Note:* Samples containing sodium azide should not be used in the assay.

### 4.1 Specimen Collection

Saliva samples should be collected only using special saliva sampling devices (vial and straw), e.g. SALI-TUBES 100 (SLV-4158). Do not use Salivette (cotton swab) for sampling.

Due to the cyclic secretion pattern of steroid hormones it is important to care for a proper timing of the sampling.

In order to avoid arbitrary results we recommend that 5 samples always be taken within a period of 2 – 3 hours (*multiple sampling*) preferably before a meal.

As food might contain significant amounts of steroid hormones samples preferably should be taken while fasting. If fasting should be a problem the collection period should be timed just before lunch or before dinner.

NOTE: The clinical significance of the Estriol determination can be invalid if the sample contains natural or synthetic steroids.

### 4.2 Specimen Storage and Preparation

The saliva samples may be stored at 2 °C to 8 °C up to one week, and should be frozen at –20 °C for longer periods. Repeated thawing and freezing should be avoided.

Each sample has to be frozen, thawed, and centrifuged at least once in order to separate the mucins by centrifugation.

Upon arrival of the samples in the lab the samples have to stay in the deep freeze at least overnight. Next morning the frozen samples are warmed up to room temperature and mixed carefully.

Then the samples have to be centrifuged for 5 to 10 minutes (at 2000 - 3000 x g).

Now the clear colorless supernatant is easy to pipette.

If a set of multiple samples is to be tested, the lab (after at least one freezing, thawing, and centrifugation cycle) has to mix the 5 single samples in a separate sampling device and perform the testing from this mixture.

### 4.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Incubation Buffer* (1+3) and re-assayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) Dilution 1+3: 10 µL saliva + 30 µL *Incubation Buffer* (mix thoroughly)

## 5 ASSAY PROCEDURE

### 5.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

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### 5.2 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **50 µL** of each ***Standard, Control*** and **samples** with new disposable tips into appropriate wells.
3. Dispense **100 µL** of diluted ***Enzyme Conjugate*** into each well. (See "Preparation of Reagents".)
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **60 minutes** at room temperature (22°C – 28°C).
5. Briskly shake out the contents of the wells.
   Rinse the wells **3 times** with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   **Important note:**
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add **100 µL** of ***Substrate Solution*** to all well.
7. Incubate for **15 minutes** at room temperature (22°C – 28°C) in the dark.
8. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to all wells. Thoroughly mix for 10 seconds.
9. Read the absorbance (OD) of each well at **450±10 nm** with a microtiter plate reader.
   It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

### 5.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

## 6 REFERENCES

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