DRG® Estradiol sensitive ELISA (EIA-4399)

研究用

Revised 20 May 2005

1 INTRODUCTION

The **DRG® Estradiol sensitive Enzyme Immunoassay Kit** provides materials for the quantitative determination of **Estradiol** in serum and plasma.
This assay is intended for in vitro diagnostic use only.

2 PRINCIPLE OF THE TEST

The DRG® **Estradiol sensitive** ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on the **Estradiol** molecule.
Endogenous **Estradiol** of a patient sample competes with a **Estradiol** horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.
The amount of bound peroxidase conjugate is reverse proportional to the concentration of **Estradiol** in the sample. After addition of the substrate solution, the intensity of colour developed is reverse proportional to the concentration of **Estradiol** in the patient sample.

3 PRECAUTIONS

- This kit is for in vitro diagnostic use only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- 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.
- Avoid contact with Stop Solution containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even if 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.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
- Safety Data Sheets for this product are available upon request directly from DRG International, Inc..
- The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

DRG

DRG® Estradiol sensitive ELISA (EIA-4399)

研究用

Revised 20 May 2005

4 KIT COMPONENTS

4.1 Contents of the Kit

1. **Microtiterwells**, 12x8 (break apart) strips, 96 wells
   Wells coated with anti- Estradiol polyclonal antibody
2. **Standard (Standard 0-4)**, 5 vials, 1 ml, ready to use
   0; 3; 10; 50; 200 pg/ml
   Conversion factor: 1 pg/ml = 3.67 pmol/L
3. **Sample Diluent**, 1 vial, 3 ml, ready to use
4. **Enzyme Conjugate**, 1 vial, 25 ml, ready to use
   Anti- Estradiol antiserum conjugated to horseradish peroxidase
5. **Substrate Solution**, 1 vial, 25 ml, ready to use
   TMB
6. **Stop Solution**, 1 vial, 14 ml, ready to use
   contains 0.5M H_2SO_4
   Avoid contact with the stop solution. It may cause skin irritations and burns.
7. **Wash Solution**, 1 vial, 30 ml (40X concentrated)
   see „Preparation of Reagents“

Note: Additional *Sample Diluent* for sample dilution is available on request.

4.1.1 Equipment and material required but not provided

- A microtiterplate calibrated reader (450±10 nm)(e.g. the DRG International Microtiterplate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Aqua dest.

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

4.3 Preparation of Reagents

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

Wash Solution
Dilute 30 ml of concentrated Wash Solution with 1170 ml deionized water to a final volume of 1200 ml.
The diluted Wash Solution is stable for 2 weeks at room temperature.

4.4 Disposal of the Kit

The disposal of the kit must be made according to the national official regulations.

DRG

DRG® Estradiol sensitive ELISA (EIA-4399)

研究用

Revised 20 May 2005

4.5 Damaged Test Kits

In case of any severe damage of the test kit or components, DRG® have to be informed written, 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.

5

Serum or plasma (EDTA-, Heparin- or citrat plasma) can be used in this assay.
Do not use haemolytic, icteric or specimen lipaemic specimens.

5.1 Specimen Collection

Serum:
Collect blood by venipuncture (e.g Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature.

Plasma:
Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.
(E.g for EDTA plasma Sarstedt Monovette – red cap - # 02.166.001; for Heparin plasma Sarstedt Monovette – orange cap - # 02.165.001; for Citrat plasma Sarstedt Monovette – green cap - # 02.167.001.)

5.2 Specimen Storage

Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying.
Specimen held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

If in an initial assay, a serum specimen is found to contain more than the highest standard, the specimens can be diluted 10-fold or 100 fold with *Sample Diluent* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) dilution 1:10: 10 µl Serum + 90 µl Sample Diluent (mix thoroughly)
b) dilution 1:100: 10 µl dilution a) 1:10 + 90 µl Sample Diluent (mix thoroughly).

6 TEST PROCEDURE

6.1 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 pipet tips for each standard, control of 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 be 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.

DRG

DRG® Estradiol sensitive ELISA (EIA-4399)

研究用

Revised 20 May 2005

6.2 Assay Procedure

All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.

1. Secure the desired number of Microtiterwells in the holder.
2. Dispense **100 µl** of each Standard and samples with new disposable tips into appropriate wells.
3. Dispense **200 µl** Enzyme Conjugate into each well.
4. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for **4 hours** at room temperature without covering the plate.
6. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted Wash Solution (400 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!
7. Add **200 µl** of Substrate Solution to each well.
8. Incubate for **30 minutes** at room temperature.
9. Stop the enzymatic reaction by adding **100 µl** of Stop Solution to each well.
10. Read the OD at **450±10 nm** with a microtiterplate reader **within 10 minutes** after adding the Stop Solution.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient 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: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log can generally give a good fit.
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.

Below is listed a typical example of a standard curve with the Estradiol sensitive ELISA.

Standard	Optical Units (450 nm)
Standard 0 (0 pg/ml)	2.03
Standard 1 (3 pg /ml)	1.83
Standard 2 (10 pg /ml)	1.38
Standard 3 (50 pg /ml)	0.63
Standard 4 (200 pg /ml)	0.30

7 EXPECTED VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

8 ASSAY CHARACTERISTICS

8.1 Assay Dynamic Range

The range of the assay is between 0 – 2 00 pg/ml.

8.2 Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross-reactivity of the assay:

Steroid	Cross-Reactivity (%)
Estradiol	100
Estron	0.2
Estriol	0.05
Androstenedione	0
Androsterone	0
Corticosterone	0
Epiandrosterone	0
16-Epiestriol	0
Estradiol-3-sulfate	0
Estradiol-3-glucuronide	0
Estradiol-17a	0
Estriol-16-glucuronide	0
Estrone-3-sulfate	0
Dehydroepiandrosterone	0
11-Desoxycortisol	0
11-Desoxycorticosterone	0
21-Desoxycortisol	0
Dihydrotestosterone	0
Dihydroepiandrosterone	0
20-Dihydroprogesterone	0
11-Hydroxyprogesterone	0
17α-Hydroxyprogesterone	0
17α-Pregnenolone	0
17α Progesterone	0
Pregnanediol	0
Pregnantriol	0
Pregnenolone	0
Progesterone	0
Testosterone	0

8.3 Analytical Sensitivity

The analytical sensitivity was calculated to be < 1 pg/ml.

8.4 Accuracy

8.4.1 Quality Control

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or DRG® directly.

9 LIMITATIONS OF USE

9.1 Interfering Substances

Any improper handling of samples or modification of this test might influence the results.

10 LEGAL ASPECTS

10.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG®.

10.2 Therapeutical Consequences

Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 10.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutical consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutical consequences.

DRG

DRG® Estradiol sensitive ELISA (EIA-4399)

研究用

Revised 20 May 2005

10.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 10.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

SYMBOLS USED WITH DRG® ELISA´S

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic	In-vitro-Diagnostikum	Diagnostic in vitro	Diagnóstico in vitro	Diagnostica in vitro
REF	Catalogue number	Katalog-Nr.	Référence	No de catálogo	No. di Cat.
LOT	Lot. No.	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
Legal Manufacturer	Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Distributeur	Distributeur	Distribuidor	Distributtore
	User´s Manual	Arbeitsanleitung	Mode d'emploi	Instrucciones de empleo	Istruzioni d'uso
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità
Microtiterwells	Microtiterwells	Mikrotiterwells	Barrettes de microtitration	Pocillos de la Microplaca	Pozzetti della micropiastra
Antiserum	Antiserum	Antiserum	Antisérum	Antisuero	Antisiero
Enzyme Conjugate	Enzyme Conjugate	Enzym Konjugat	Conjugué enzymatique	Conjugado enzimático	Tracciante enzimatico
Enzyme Complex	Enzyme Complex	Enzym Komplex	Complex enzymatique	Complex enzimático	Complesso enzimatico
Substrate Solution	Substrate Solution	Substrat Lösung	Solution substrat	Solución de sustrato	Soluzione di substrato
Stop Solution	Stop Solution	Stopplösung	Solution d'arret	Solución de paro	Soluzione di arresto
Zero Standard	Zero Standard	Nullstandard	Standard 0	Standard 0	Standard 0
Standard	Standard	Standard	Standard	Calibrador	Calibratore
Control	Control	Kontrolle	Controle	Control	Controllo
Assay Buffer	Assay Buffer	Assay Puffer	Tampon d'essai	Tampón de ensayo	Tampone del test
Wash Solution	Wash Solution	Waschlösung	Solution de lavage	Solución de lavado	Soluzione di lavaggio

DRG

DRG® Estradiol sensitive ELISA (EIA-4399)

研究用

Revised 20 May 2005

1N NaOH	1N NaOH	1N NaOH	1N NaOH	1N NaOH	1N NaOH (idrossido di sodio 1N)
Sample Diluent	Sample Diluent	Probenverdünnungsmedium			
Conjugate Diluent	Conjugate Diluent	Konjugatverdünnungsmedium			