DRG® Cortisol ELISA (EIA-1887)

REVISED 28 JULY 2010 RM (VERS. 1.1) **USA: RUO**

INTRODUCTION

The **DRG Cortisol Enzyme Immunoassay Kit** provides materials for the determination of Cortisol in serum and plasma.

This assay is intended for research use only.

PRINCIPLE OF THE TEST

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

PRECAUTIONS

- This kit is for Research 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 and microtiterplate readers.
- 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.
- 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.

KIT COMPONENTS

Contents of the Kit

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with a anti-Cortisol antibody (monoclonal).
2. ***Standard (Standard 0-6)*****,** 7 vials, 1 mL, ready to use;
   Concentrations: 0, 20, 50, 100, 200, 400, 800 ng/mL,
   thus corresponding to 0, 55.2, 138, 276, 552, 1104, 2208 nmol/L.
   Conversion factor: 1 ng/mL = 2.76 nmol/l.)
   contain 0.3% Proclin as a preservative
3. ***Enzyme Conjugate***, 1 vial, 25 mL, ready to use;
   Cortisol conjugated to horseradish Peroxidase;
   contains 0.3% Proclin as a preservative.
4. ***Substrate Solution***, 1 vial, 14 mL, ready to use;
   Tetramethylbenzidine (TMB).
5. ***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.
6. ***Wash Solution***, 1 vial, 30 mL (40X concentrated);
   see "Preparation of Reagents".

Note: Additional *Standard 0* for sample dilution is available upon request.

Equipment and material required but not provided

- A microtiter plate calibrated reader (450±10 nm), (e.g. the DRG International Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled water

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.

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.

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).

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.

SPECIMEN

Serum or plasma (EDTA-, Heparin- or citrate plasma) can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

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. Do not centrifuge before complete clotting has occurred. Donors receiving anticoagulant therapy may require increased clotting time.

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 Citrate plasma Sarstedt Monovette – green cap - # 02.167.001.)

Specimen Storage

Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying.
Specimens 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.

Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* 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 *Standard 0* (mix thoroughly)
b) Dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

TEST PROCEDURE

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.

Assay Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense **20 µL** of each *Standard, Control* 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 **60 minutes** 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 **100 µL** of *Substrate Solution* to each well.
8. Incubate for **15 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 microtiter plate reader **within 10 minutes** after adding the *Stop Solution*.

Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and donor 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. 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.

Below is listed a typical example of a standard curve with the DRG® Cortisol ELISA.

Standard	Optical Units (450 nm)
Standard 0 (0 ng/mL)	2.30
Standard 1 (20 ng/mL)	1.67
Standard 2 (50 ng/mL)	1.24
Standard 3 (100 ng/mL)	0.87
Standard 4 (200 ng/mL)	0.57
Standard 5 (400 ng/mL)	0.35
Standard 6 (800 ng/mL)	0.23

REFERENCES

1. L. Thomas, Labor und Diagnose, 4. Auflage, 1992
2. Tietz, N.W., Textbook of Clinical Chemistry, Saunders, 1968