DRG® Hepcidin Prohormone ELISA (EIA-4644)

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1 INTRODUCTION

The DRG Hepcidin Prohormone Enzyme Immunoassay Kit provides materials for determination of Hepcidin Prohormone in human serum and urine.

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

2 PRINCIPLE OF THE TEST

The DRG Hepcidin Prohormone 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 polyclonal antibody directed towards an antigenic site on the Hepcidin Prohormone molecule (28-47 aa). Endogenous Hepcidin Prohormone of a sample competes with a Hepcidin Prohormone-biotin conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.
The amount of bound biotin conjugate is reverse proportional to the concentration of Hepcidin Prohormone in the sample.
After addition of the substrate solution, the intensity of colour developed is reverse proportional to the concentration of Hepcidin Prohormone in the sample.

3 PRECAUTIONS

- This kit is intended for Research Use Only. Not for use in diagnostic procedures.
- 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.

4 KIT COMPONENTS

4.1 Contents of the Kit

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with anti Pro-Hepcidin antibody (polyclonal).
2. ***Standard (Standard 0-6)***, 7 vials (lyophilized), 1 mL;
   Concentrations: 10, 50, 100, 250, 500, 1000 ng/mL of synthetic peptide Hepcidin (28-47).
   Contains < 0.02% methylisothiazolone and < 0.02% bromonitrodioxane as preservative.
   See „Preparation of Reagents“;
3. ***Control***, 1 vial (lyophilized), 1 mL,
   Control value and range please refer to vial label or QC-Datasheet.
   see „Reagent Preparation“
   Contains < 0.02% methylisothiazolone and < 0.02% bromonitrodioxane as preservative.
4. ***Assay Buffer***, 1 vial, 14 mL, ready to use,
   contains < 0.02% methylisothiazolone and < 0.02% bromonitrodioxane as preservative.
5. ***Biotin Conjugate***, 1 vial, 14 mL, ready to use,
   Pro-Hepcidin fragment conjugated to biotin;
   contains < 0.02% methylisothiazolone and < 0.02% bromonitrodioxane as preservative.
6. ***Enzyme Complex***, 1 vial, 14mL, ready to use,
   contains horseradish Peroxidase,
   contains < 0.02% methylisothiazolone and < 0.02% bromonitrodioxane as preservative.
7. ***Substrate Solution***, 1 vial, 14 mL, ready to use;
   Tetramethylbenzidine (TMB).
8. ***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.
9. ***Wash Solution***, 1 vial, 30 mL (40X concentrated);
   see „Preparation of Reagents“.

4.1.1 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.
- 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. 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.
Opened kits retain activity for two months if stored as described above.

4.3 Preparation of Reagents

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

Standards

Reconstitute the lyophilized contents of the standard vial with 1.0 mL Aqua dest.
Note: *The reconstituted standards are stable for 6 days at 2-8°C. For longer storage freeze at -20°C.*

Control

Reconstitute the lyophilized content with 1.0 mL Aqua dest, and let stand for 10 minutes in minimum. Mix the control several times before use.
Note: *The reconstituted control is stable for 6 days at 2-8°C. For longer storage freeze at -20°C.*

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 regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

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 SPECIMEN

Serum and urine can be used in this assay.
Do not use haemolytic, icteric or lipemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

No special handling is necessary for urine samples.

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 2500 x g for 10 min at 4°C. Aliquots should be stored at –20°C.

5.2 Specimen Storage

Specimens should be capped and may be stored for up to 24 hours 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.

Note: *Samples which were frozen for a longer period or which were repeatedly thawed show higher Pro-Hepcidin concentrations than freshly collected samples which were frozen only once.*

5.3 Specimen Dilution

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

6.2 Assay Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense **100 µl** of *Assay Buffer* into each wells.
3. Dispense **50 µl** of each *Standard, Control* and samples with new disposable tips into appropriate wells.
4. Dispense **100 µl** *Biotin Conjugate* into each well.
5. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
6. Incubate for **120 minutes** at room temperature (without covering the plate).
7. Briskly shake out the contents of the wells.
   Rinse the wells 5 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!
8. Add **100 µl** of *Enzyme Complex* to each well.
9. Incubate for **60 minutes** at room temperature.
10. Briskly shake out the contents of the wells.
    Rinse the wells 5 times with diluted *Wash Solution* (400 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets.
11. Add **100 µl** of *Substrate Solution* to each well.
12. Incubate for **30 minutes** at room temperature.
13. Stop the enzymatic reaction by adding **100 µl** of *Stop Solution* to each well.
14. Read the OD at **450±10 nm** with a microtiter plate reader **within 10 minutes** after adding the *Stop Solution*.

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6.3 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. 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 Hepcidin Prohormone ELISA.

Standard	Optical Units (450 nm)
Standard 0 (0 ng/mL)	1.85
Standard 1 (10 ng/mLl)	1.72
Standard 2 (50 ng/mL)	1.38
Standard 3 (100 ng/mL)	1.16
Standard 4 (250 ng/mL)	0.82
Standard 5 (500 ng/mL)	0.63
Standard 6 (1000 ng/mL)	0.48

6.4 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.

7 REFERENCES

1. Kulaksiz H, et al. Pro-hepcidin: expression and cell-specific localization in the liver and its regulation in hereditary hemochromatosis, chronic renal insufficiency, and renal anemia. GUT 2004; 53: 735-743
2. Krause A, Neitz S, Magert HJ, et al. LEAP-1, a novel highly-disulfide bonded human peptide, exhibits antimicrobial activity. FEBS Lett 2000; 480; 147-150
3. Park CH, Valore EV, Waring AJ, et al. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem 2001; 276; 7806-7810