



## User's Manual

# Hepcidin ELISA Kit



DEIA2022



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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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## PRODUCT INFORMATION

### Intended Use

The Hepcidin ELISA is an enzyme immunoassay for measurement of Hepcidin in serum.

### Principles of Testing

The Hepcidin 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 the antigenic site of the bioactive Hepcidin 25 molecule. Endogenous Hepcidin of a patient sample competes with the added Hepcidin-biotin conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. An incubation with a streptavidin-peroxidase enzyme complex and a second wash step follows. The addition of substrate solution results in a colour development which is stopped after a short incubation. The intensity of colour developed is reverse proportional to the concentration of Hepcidin in the patient sample.

### Reagents And Materials Provided

1. Microtiterwells, 12x8 (break apart) strips, 96 wells;  
Wells coated with anti-Hepcidin antibody (monoclonal).
2. Standard (Standard 0-5), 6 vials, (lyophilized) 0.5 mL;  
Concentrations: 0.0 – 3.85 – 11.5 – 23 – 70 – 140.0 ng/mL synthetic Hepcidin.
3. Control low and high, 2 vials (lyophilized) 0.5 mL;  
For control values and ranges please refer to vial label or QC-Datasheet.
4. Sample Buffer, 3 mL, ready to use
5. Assay Buffer, 1 vial, 25 mL, ready to use,
6. Enzyme Conjugate, 1 vial, 14 mL, ready to use, biotinylated Hepcidin fragment
7. Enzyme Complex, 1 vial, 14 mL, ready to use, contains streptavidin peroxidase
8. Substrate Solution, 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).
9. Stop Solution, 1 vial, 14 mL, ready to use, contains 0.5M H<sub>2</sub>SO<sub>4</sub>,  
Avoid contact with the stop solution. It may cause skin irritations and burns.
10. Wash Solution, 1 vial, 30 mL (40X concentrated)

Note: Additional assay Buffer for sample dilution is available upon request.

### Materials Required But Not Supplied

1. A microtiter plate calibrated reader (450 ± 10 nm)
2. Calibrated variable precision micropipettes.
3. Absorbent paper.

4. Distilled or deionized water
5. Timer
6. Semi logarithmic graph paper or software for data reduction

## Storage

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

Opened reagents must be stored at 2°C to 8°C. Microtiter wells must be stored at 2°C to 8°C. Once the foil bag has been

opened, care should be taken to close it tightly again.

Opened kits retain activity for two weeks if stored as described above.

## Specimen Collection And Preparation

Only serum 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. Patients receiving anticoagulant therapy may require increased clotting time.

### Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 24 hours at 2 °C to 8 °C prior to assaying.

Specimens held for a longer time should be frozen only once at  $\leq -20^{\circ}\text{C}$  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 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  $\mu\text{L}$  Serum + 90  $\mu\text{L}$  Assay Buffer (mix thoroughly)
- b) dilution 1:100: 10  $\mu\text{L}$  dilution a) 1:10 + 90  $\mu\text{L}$  Assay Buffer (mix thoroughly).

## Reagent Preparation

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

#### Standards

Reconstitute the lyophilized contents of the standard vials with 0.5 mL distilled water.

Note: The reconstituted standards are stable for 1 week at 2 °C to 8 °C. For longer storage freeze at -20°C.

#### Control

Reconstitute the lyophilized content with 0.5 mL distilled water and let stand for 10 minutes in minimum.

Mix the control several times before use.

Note: The reconstituted control is stable for 1 week at 2 °C to 8 °C. For longer storage freeze at -20°C.

#### Wash Solution

Add deionized water to the 40X concentrated 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.

## Assay 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.

#### Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Add 10 µL Sample Buffer to each of this wells.
3. Dispense 20 µL of each Standard, Control and samples with new disposable tips into appropriate wells.
4. Incubate for 30 minutes at room temperature on a plate shaker at  $\approx$  500 rpm.
5. Add 150 µL Assay Buffer and add 100 µL Enzyme Conjugate to each of this wells.
6. Incubate for 180 minutes at room temperature on a plate shaker at  $\approx$  500 rpm.
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. Dispense 100 µL Enzyme Complex into each well.

9. Incubate for 45 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. Determine the absorbance (OD) of each well at  $450 \pm 10$  nm with a microtiter plate reader.

It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

## Calculation

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using semi-logarithmic graph paper, 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 or reported as > 140 nmol/mL. For the calculation of the concentrations this

dilution factor has to be taken into account.

## Typical Standard Curve

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

## Precautions

1. 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.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
4. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
5. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
6. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
7. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
8. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the readings of the assay. However, values for the patient samples will not be affected.
9. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
10. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
11. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
12. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
13. Do not use reagents beyond expiry date as shown on the kit labels.
14. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
15. 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.
16. Avoid contact with Stop Solution containing 0.5 M H<sub>2</sub>SO<sub>4</sub>. It may cause skin irritation and burns.
17. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.



18. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
19. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.

