Hepcidin-25 ELISA Kit (Human) (OKBA00029) Instructions for use

For the Quantitative Determination of Hepcidin-25 in Human Serum or Plasma

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES

PRINCIPLE OF THE ASSAY

The Hepcidin-25 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 (mouse) antibody directed towards an antigenic site of the Hepcidin-25 molecule.

Endogenous Hepcidin-25 of a sample competes with a Hepcidin-25-biotin conjugate (Enzyme Conjugate) for binding to the coated antibody. After 60 minutes incubation at room temperature, the microtiter plate is washed to remove unbound Hepcidin-25-biotin conjugate and stop the competition reaction.

In the following 30 minutes incubation at room temperature, the bound biotin molecules are detected with streptavidin peroxidase (Enzyme Complex). After incubation the plate is washed again.

After addition of the substrate solution and 20 minutes incubation, the intensity of color developed is inversely proportional to the concentration of Hepcidin-25 in the sample.

MATERIALS PROVIDED

1. ***Microtiter wells***, 12 x 8 (removable) strips, 96 wells; Wells coated with anti-Hepcidin-25 antibody (monoclonal).
2. ***Standard***, 6 vials, lyophilized, 0.5 mL; Concentrations: 0–1–3–9–27–81 ng/mL Conversion: 1 ng/mL = 0.358 nmol/L See "Reagent Preparation". Contain non-mercury preservative.
3. ***Control Low & High***, 2 vials, lyophilized, 0.5 mL; For control values and ranges please refer to vial label. See "Reagent Preparation". Contains a non-mercury preservative.
4. ***Sample Diluent***, 1 vial, 3mL, ready to use; Contains non-mercury preservative
5. ***Enzyme Conjugate***, 1 vial, 7 mL, ready to use, Hepcidin-25 conjugated to biotin; Contains non-mercury preservative.
6. ***Enzyme Complex***, 1 vial, 14 mL, ready to use, Streptavidin conjugated to horseradish peroxidase Contains non-mercury preservative.
7. ***Substrate Solution***, 1 vial, 14mL, ready to use, Tetramethylbenzidine (TMB).
8. ***Stop Solution***, 1 vial, 14mL, ready to use, contains 0.5 M 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 "Reagent Preparation".

MATERIALS REQUIRED BUT NOT PROVIDED

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. Semilogarithmic graph paper or software for data reduction

STORAGE CONDITIONS

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 8 weeks if stored as described above.

REAGENT PREPARATION

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

Standards

Reconstitute the lyophilized contents of each standard vial with 0.5 mL deionized water and let stand for at least 10 minutes. Mix several times before use.

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

Controls

Reconstitute the lyophilized content with 0.5 mL deionized water and let stand for at least 10 minutes. Mix the control several times before use.

Note: *The reconstituted controls are stable for 2 months at 2 °C - 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 (18-25ºC).

SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (EDTA-, heparin- or citrate plasma) can be used in this assay.

Do not use hemolytic, icteric or lipemic 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 for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Samples containing anticoagulant may require increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 4 days at 2 °C to 8 °C prior to assaying.

Specimens held for a longer time (up to 12 months) 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 *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 sample + 90 µL *Sample Diluent* (mix thoroughly)

b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Sample Diluent*
(mix thoroughly).

ASSAY PROCEDURE

General Remarks

1. All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
2. Once the test has been started, all steps should be completed without interruption.
3. Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
4. 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.
5. 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. Dispense **20 µL** of each ***Standard, Control*** and **samples** with new disposable tips into appropriate wells.
3. Dispense **50 µL** ***Enzyme Conjugate*** into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **60 minutes** at room temperature.
5. Briskly shake out the contents of the wells. Rinse the wells **4 x** with **400 µL diluted *Wash Solution*** per well (if a plate washer is used) - or
   4 x with **300 µL diluted *Wash Solution*** per well for manual washing.
   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. Dispense **100µL** of ***Enzyme Complex*** into appropriate wells.
7. Incubate for **30 minutes** at room temperature.
8. Briskly shake out the contents of the wells. Rinse the wells **4 x** with **400 µL diluted *Wash Solution*** per well (if a plate washer is used) - or.
   4 x with **300 µL diluted *Wash Solution*** per well for manual washing.o
   Strike the wells sharply on absorbent paper to remove residual droplets.
9. Add **100 µL** of ***Substrate Solution*** to each well.
10. Incubate for **20 minutes** at room temperature.
11. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
12. Determine 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*.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and 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 Instructions for Use have been calculated automatically using a 4-Parameter curve fit.(4ParameterRodbardor4 ParameterMarquardtarethe preferred methods.) 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 > 81 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

EXAMPLE OF TYPICAL STANDARD CURVE

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

Standard	Optical Units (450 nm)
Standard 0 (0 ng/mL)	2.03
Standard 1 (1 ng/0mL)	1.70
Standard 2 (3 ng/mL)	1.29
Standard 3 (9 ng/mL)	0.77
Standard 4 (27 ng/mL)	0.37
Standard 5 (81 ng/mL)	0.17

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Technical Support:

For optimal service, please be prepared to supply the lot number of the kit used.

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