DATA SHEET

Human Hepcidin(Hepc) ELISA Kit

Catalog No. CSB-E13062h

(96 tests)

- This immunoassay kit allows for the in vitro quantitative determination of **human Hepc** concentrations in **serum, plasma**.
- **Expiration date** **six** months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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INTRODUCTION

Human hepcidin, a 25–amino acid peptide made by hepatocytes, may be a new mediator of innate immunity and the long-sought iron-regulatory hormone. The synthesis of hepcidin is greatly stimulated by inflammation or by iron overload. Evidence from transgenic mouse models indicates that hepcidin is the predominant negative regulator of iron absorption in the small intestine, iron transport across the placenta, and iron release from macrophages. The key role of hepcidin is confirmed by the presence of nonsense mutations in the hepcidin gene, homozygous in the affected members, in 2 families with severe juvenile hemochromatosis. Recent evidence shows that deficient hepcidin response to iron loading may contribute to iron overload even in the much milder common form of hemochromatosis, from mutations in the HFE gene. In anemia of inflammation, hepcidin production is increased up to 100-fold and this may account for the defining feature of this condition, sequestration of iron in macrophages. The discovery of hepcidin and its role in iron metabolism could lead to new therapies for hemochromatosis and anemia of inflammation.

PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with an antibody specific to Hepc. Standards or samples are then added to the appropriate microtiter plate wells with a Horseradish Peroxidase (HRP) -conjugated antibody preparation specific for Hepc and incubated. Then substrate solutions are added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of Hepc in the samples is then determined by comparing the O.D. of the samples to the standard curve.

DETECTION RANGE

12.5 ng/ml-400 ng/ml. The standard curve concentrations used for the ELISA's were 400 ng/ml, 200 ng/ml, 87.5 ng/ml, 37.5 ng/ml, 12.5ng/ml.

SPECIFICITY

This assay recognizes human Hepc. No significant cross-reactivity or interference was observed.

SENSITIVITY

The minimum detectable dose of human Hepc is typically less than 6.25 ng/ml.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate	1
Standard(S0-S5)	6 x 0.5 ml
HRP-conjugate	1 x 6 ml
Substrate A	1 x 7 ml
Substrate B	1 x 7 ml
Stop Solution	1 x 7 ml

Standard	S0	S1	S2	S3	S4	S5
Concentration (ng/ml)	0	12.5	37.5	87.5	200	400

STORAGE

1. Unopened test kits should be stored at 2-8 C upon receipt and the microtiter plate should be kept in a sealed bag. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.

2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.

SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20℃. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

- **Plasma** Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20℃. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate. All the reagents should be added directly to the liquid level in the well. The pipette should avoid contacting the inner wall of the well.

1. Set a Blank well without any solution. Add 50µl of **Standard** or **Sample** per well.
2. Add 50µl of **HRP-Conjugate** to each well (Not to Blank!). Incubate for 1 hour at 37°C.
3. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with **ddH_2O** (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good

performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 50µl of **Substrate A** and 50µl **Substrate B** to each well. Incubate for 15 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
5. Add 50µl of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
6. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting

the log of the Hepc concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

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