abbexa®

Rat Hepcidin 25 (Hepc25) ELISA Kit

Catalog No.: abx055501

Size: 96T

Range: 26 pg/ml - 1500 pg/ml

Sensitivity: 5 pg/ml

Storage: Store at 2-8°C for 6 months.

Application: For quantitative detection of Hepc25 in Rat Serum, Plasma, Cell Culture Supernatants and tissue homogenates.

Introduction: Hepcidin is a protein that in humans is encoded by the HAMP gene. Hepcidin is a key regulator of the entry of iron into the circulation in mammals. Hepcidin is a regulator of iron metabolism. Hepcidin inhibits iron transport by binding to the iron export channel ferroportin which is located on the basolateral surface of gut enterocytes and the plasma membrane of reticuloendothelial cells (macrophages). Hepcidin ultimately breaks down the transporter protein in the lysosome. Inhibiting ferroportin prevents iron from being exported and the iron is sequestered in the cells. By inhibiting ferroportin, hepcidin prevents enterocytes from allowing iron into the hepatic portal system, thereby reducing dietary iron absorption. The iron release from macrophages is also reduced by ferroportin inhibition. Increased hepcidin activity is partially responsible for reduced iron availability seen in anemia of chronic inflammation, such as renal failure.

Principle of the Assay

This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. Anti-Hepc25 antibody is pre-coated onto a 96-well plate. An HRP conjugated antibody specific to Hepc25 is used as detection antibody. The standards, test samples and HRP conjugate reagent are added to the wells and incubated. Unbound conjugates are washed away with wash buffer. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the Hepc25 amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of Hepc25 can be calculated.

Kit components

1. One pre-coated 96-well microplate (8 × 12 well strips)
2. Standard: 0.5 ml
3. Standard diluent buffer: 1.5 ml
4. Wash buffer (30X): 20 ml. Dilution: 1/30
5. Sample diluent buffer: 6 ml
6. HRP conjugate reagent: 6 ml
7. Stop solution: 6 ml
8. TMB substrate A: 6 ml
9. TMB substrate B: 6 ml
10. Plate sealer: 2
11. Hermetic bag: 1

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare standard/sample dilutions
7. Deionized or distilled water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting and analyze immediately (within 2 hours) or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 2000-3000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using citrate or EDTA as an anticoagulant. Centrifuge for 20 minutes at 2000-3000 × g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Cell culture supernatant:** Centrifuge at approximately 2000-3000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.
- **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.

Note:

- » The QC data has been produced using a 1:5 dilution with the provided Sample diluent buffer. If the sample concentration is unknown, we would recommend a starting point of 1:5 dilution with the provided Sample diluent buffer.
- » Samples must be diluted so that the expected concentration falls within the kit's range.
- » Fresh samples or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.
- » Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used.
- » NaN_3 cannot be used as test sample preservative, since it inhibits HRP.
- » Always use non-pyrogenic, endotoxin-free tubes for blood collection.

2. Wash buffer

Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

3. Standard

Label 5 tubes with 1200 pg/ml, 800 pg/ml, 400 pg/ml, 200 pg/ml and 100 pg/ml respectively. Aliquot 0.2 ml of the Standard diluent buffer into the first two tubes labeled 1200 pg/ml and 800 pg/ml respectively and 0.3 ml of the Standard diluent buffer into each remaining tube. Add 0.4 ml of 1800 pg/ml standard solution into 1st tube and mix thoroughly. Transfer 0.4 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on. The standard solutions are best used within 2 hours. Avoid repeated freeze-thaw cycles.
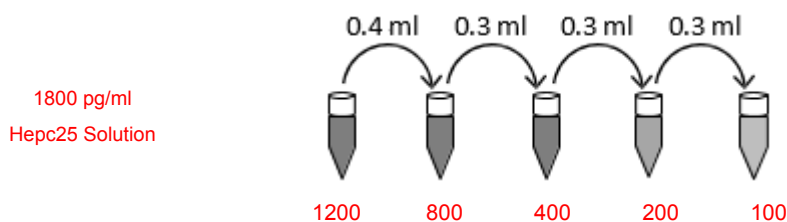
B. Assay Procedure

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, record their positions. It is recommended to assay standards, samples and controls in duplicate.

2. Aliquot 50 µl of the diluted standards into the standard wells.

3. Aliquot 50 µl of Standard diluent buffer into the control (zero) well. Do not add sample and HRP conjugate reagent into the control (zero) well.

4. Aliquot 50 µl of appropriately diluted sample (Rat serum, plasma or cell culture supernatants) into the test sample wells. Add the solution at the bottom without touching the side walls of the well. Shake the plate mildly to mix the contents.

5. Seal the plate with a cover and incubate at 37°C for 30 min.

6. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. Fill each well completely with Wash buffer (300µL) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.

7. Aliquot 50 µl of HRP conjugate reagent into each well (except control well).

8. Seal the plate with a cover and incubate at 37°C for 30 min.

9. Remove the cover and repeat the aspiration/wash process 5 times as explained in step 6.

10. Aliquot 50 µl of TMB Substrate A into each well and 50 µl of TMB Substrate B. Vortex the plate gently on an ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds). Cover the plate and incubate at 37°C for 15 min. Avoid exposure to light.

11. Add 50 µl of Stop solution into each well. There should be a color change to yellow. Gently tap the plate to ensure thorough mixing.

12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

For calculation, (relative O.D.450) = (O.D.450 of each well) – (O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). Log-log curve fitting is recommended for data analysis. The Rat Hepc25 concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

1. Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.

2. Avoid foaming or bubbles when mixing or reconstituting components.

3. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.

4. It is recommend to measure each standard and sample in duplicate or triplicate.

5. Do NOT let the plate dry out completely as this will inactivate the biological material on the plate.

6. Ensure plates are properly sealed or covered during incubation steps.

7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.

8. To avoid cross contamination do not reuse pipette tips and tubes.

9. Do not use expired components or components from a different kit.

10. Store the TMB substrate B in the dark and to avoid edge effect of plate incubation for temperature differences it is recommended to equilibrate the TMB substrates at room temperature prior to use. TMB Substrate solution is easily contaminated; sterility precautions should be taken. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of Hepc25 were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of Hepc25 were tested on 3 different plates, 8 replicates in each plate.

CV (%) = (Standard Deviation / mean) × 100

Intra-Assay: CV<10%

Inter-Assay: CV<12%