



Rat Hemojuvelin ELISA Kit

Enzyme Immunoassay for the quantification of Rat Hemojuvelin in Rat Serum, plasma (heparin, EDTA) and cell culture supernatants.

Catalog number: ARG81907

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INTRODUCTION

The product of this gene is involved in iron metabolism. It may be a component of the signaling pathway which activates hepcidin or it may act as a modulator of hepcidin expression. It could also represent the cellular receptor for hepcidin. Alternatively spliced transcript variants encoding different isoforms have been identified for this gene. Defects in this gene are the cause of hemochromatosis type 2A, also called juvenile hemochromatosis (JH). JH is an early-onset autosomal recessive disorder due to severe iron overload resulting in hypogonadotrophic hypogonadism, hepatic fibrosis or cirrhosis and cardiomyopathy, occurring typically before age of 30. [provided by RefSeq, Jul 2008]

Acts as a bone morphogenetic protein (BMP) coreceptor. Through enhancement of BMP signaling regulates hepcidin (HAMP) expression and regulates iron homeostasis. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Hemojuvelin has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Hemojuvelin present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for Hemojuvelin is added to each well and incubated. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Hemojuvelin bound in the initial

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step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm \pm 2nm. The concentration of Hemojuvelin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Expiration date: Six months at 4°C and twelve months at -20°C

| Component | Quantity | Storage information |
|---------------------------------------|----------------------|---|
| Antibody-coated microplate | 8 X 12 strips | 4°C. Unused strips should be sealed tightly in the air-tight pouch. |
| Standard (Lyophilized) | 2 X 10 ng/vial | 4°C |
| Standard/Sample diluent | 30 ml (Ready to use) | 4°C |
| Antibody conjugate concentrate (100X) | 1 vial (130 μ l) | 4°C |
| Antibody diluent buffer | 12 ml (Ready to use) | 4°C |
| HRP-Streptavidin concentrate (100X) | 1 vial (130 μ l) | 4°C |
| HRP-Streptavidin diluent buffer | 12 ml (Ready to use) | 4°C |
| Wash Buffer (Powder) | 1 package | 4°C |
| TMB substrate | 10 ml (Ready to use) | 4°C (Protect from light) |
| STOP solution | 10 ml (Ready to use) | 4°C |
| Plate sealer | 4 strips | Room temperature |

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 610-650 nm as the reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
- The TMB Color developing agent should be colorless and transparent before using.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Do not let strips dry, as this will inactivate active components in wells.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.

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- Change pipette tips between the addition of different reagent or samples.
- Avoid using reagents from different batches.
- In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the 1X HRP-Streptavidin Solution and TMB substrate be pre-warmed in 37°C for 20-30 min before use.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1000 x g and aliquot & store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

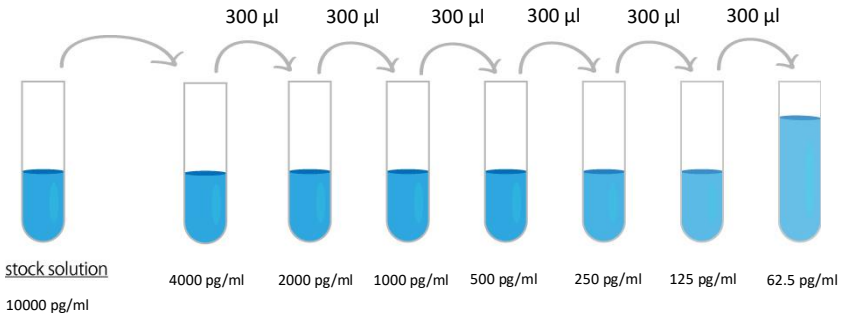
- **1X Wash Buffer:** Dissolve the wash buffer powder in 50 ml of deionized water to yield a 20X wash buffer stock. Dilute 20X wash buffer with distilled water to yield 1X wash buffer. The pH value of dissolved 1X wash buffer should be between pH7.2 to pH7.6. The 20X wash buffer stock is stable for 1-3 months at 2°C to 8°C and the dissolved 1X wash buffer is stable for a week at 2°C to 8°C.
- **1X Antibody conjugate:** It is recommended to prepare this reagent immediately prior to use and use it within 2 hours after preparation. Dilute 100X antibody conjugate concentrate into Antibody diluent buffer to yield 1X detection antibody solution. (e.g. 10 µl of 100X antibody conjugate concentrate + 990 µl of Antibody diluent buffer)
- **1X HRP-Streptavidin Solution:** It is recommended to prepare this reagent immediately prior to use and use it within 1 hour after preparation. Dilute 100X HRP-Streptavidin concentrate solution into HRP-Streptavidin diluent buffer to yield 1X HRP-Streptavidin Solution buffer. (e.g. 10 µl of 100X HRP-Streptavidin concentrate solution + 990 µl of HRP-Streptavidin diluent buffer)
- **Sample:** If the initial assay found samples contain Hemojuvelin higher than the highest standard, the samples can be diluted with Standard/Sample diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with the diluent buffer before assay.

(It is recommended to do pre-test to determine the suitable dilution)

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factor).

- **Standards:** Standard solution should be prepared within 2 hours prior to the experiment. Reconstitute the standard with 1 ml Standard/Sample diluent to yield a stock concentration of 10000 pg/ml. Allow the stock standard to sit for at least 10 minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The Standard/ Sample diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Standard/ Sample diluent as according to the suggested concentration below: 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml.
Note: The reconstituted standard solutions are best used within 2 hours. The 10 ng/ml stock standard solution should be stored at 4°C for up to 12 hours, or aliquot & store at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles



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Dilute Hemojuvelin standard as according to the table below:

| Standard | Hemojuvelin Conc. (pg/ml) | μ l of Standard/Sample diluent | μ l of standard |
|----------|---------------------------|------------------------------------|-------------------------|
| S7 | 4000 pg/ml | 600 | 400 (10000 pg/ml Stock) |
| S6 | 2000 pg/ml | 300 | 300 (S7) |
| S5 | 1000 pg/ml | 300 | 300 (S6) |
| S4 | 500 pg/ml | 300 | 300 (S5) |
| S3 | 250 pg/ml | 300 | 300 (S4) |
| S2 | 125 pg/ml | 300 | 300 (S3) |
| S1 | 62.5 pg/ml | 300 | 300 (S2) |
| S0 | 0 | 300 | 0 |

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) or 37°C before use. The 1X HRP-Streptavidin Solution and TMB substrate must be kept warm at 37°C before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard Hemojuvelin detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of Hemojuvelin amount in samples. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 μ l of standards, samples and zero controls (S0, Standard/Sample diluent) into wells. Cover the plate and incubate for 90 minutes at 37°C.
3. Aspirate each well. Complete removal of liquid by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry

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at any time. Wash step is not necessary in this step.

4. Add 100 μ l 1X Antibody conjugate into each well, gently tap the plate to mix well. Cover wells and incubate for 60 minutes at 37°C.
5. Aspirate each well and wash, repeating the process two times for a total three washes. Wash by filling each well with Wash Buffer (0.01M PBS or TBS) (300 μ l) using a squirt bottle, manifold dispenser, or autowasher, keep the wash buffer in the wells for 1 min before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry at any time.
6. Add 100 μ l of 1X HRP-Streptavidin solution to each well, gently tap the plate to mix well. Cover wells and incubate for 30 minutes at 37°C.
7. Aspirate each well and wash, repeating the process four times for a total five washes. Wash by filling each well with Wash Buffer (0.01M PBS or TBS) (300 μ l) using a squirt bottle, manifold dispenser, or autowasher, keep the wash buffer in the wells for 1 min before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry at any time.
8. Add 90 μ l of TMB substrate to each well. Incubate for 25-30 minutes at 37°C in dark. (Note: The incubation time is for reference only, the optimal incubation time should be determined by end user. And the shades of blue color can be seen in the wells with the four most concentrated

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Hemojuvelin standard solutions; the other wells show no obvious color).

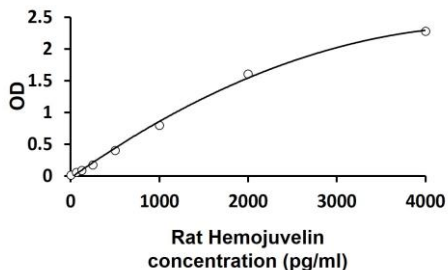
9. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing
10. Read the OD with a microplate reader at 450nm immediately. It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear 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. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

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.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Rat Hemojuvelin ranged from 62.5 pg/ml- 4000 pg/ml. The mean MDD was 31.25 pg/ml.

Specificity

This assay recognizes natural and recombinant Rat Hemojuvelin. No significant cross-reactivity or interference with the factors below was observed:

There is no detectable cross-reactivity with other relevant proteins.

Intra-assay and Inter-assay precision

The CV values of intra-assay was 5.5% and inter-assay was 6.5%.