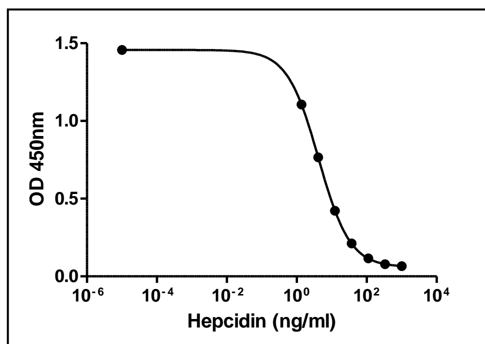
Standard Curve:

Eight standard points, including a zero, are used for each run. A typical standard curve is shown below:



Hepc-1 (ng/ml)	OD 450nm
1000	0.065
333.3	0.078
111.1	0.115
37	0.212
12.3	0.422
4.1	0.765
1.4	1.106
0.0	1.457

Quality Control:

It is recommended that each laboratory establish the range of hepc-1 controls to validate the performance of the reagents.

Calculating Results:

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit (Recommended: Graphpad Prism Software, www.graphpad.com). There is no need to correct the OD values for background. Calculate the concentration of hepcidin-1 corresponding to the mean absorbance from the standard curve. Since samples were diluted, the concentration extrapolated from the standard curve must be multiplied by the dilution factor. Example for a 5% sample: 12 µl sample / 240 µl assay volume = 1/20, thus dilution factor = 20.

Converting Results:

Results are expressed in ng/ml. To convert to nmol/L, multiply results by 0.362.
Example: 100ng/ml = 36.2nmol/L

Citations:

1. Park C.H., Valore E.V., et al (2001). The Journal of Biological Chemistry 276:7806-7810.
2. Pigeon C., Ilyin G., et al (2001). The Journal of Biological Chemistry 276:7811-7819.
3. Nicolas G., Bennoun M., et al (2001). PNAS 98: 8780-8785.
4. Ganz T. (2005). Best Practice & Research. Clinical Haematology 18: 171-182.
5. Nemeth E., Rivera S., et al (2004). The Journal of Clinical Investigation 113:1271-1276.
6. Rivera S., Nemeth E., et al (2005). Blood 106: 2196-2199.
7. Gutschow P., Schmidt P., et al (2015). Haematologica 100(2): 167-177.

SKU# HMC-001
For orders and inquires, please contact:

Intrinsic LifeSciences
505 Coast Blvd South, La Jolla, CA, 92037 Ste. 408
Tel (858) 459-1758, Fax (858) 459-3794
www.intrinsiclifesciences.com

Hepcidin-Murine Compete™ ELISA

SKU# HMC-001

Intended Use:

The Intrinsic LifeSciences Hepcidin-Murine Compete™ ELISA is designed for specific quantification of hepcidin-1 (hepc-1) in murine serum and urine. Murine hepcidin-2 (hepc-2) is not detected and does not interfere with hepc-1 measurements using this ELISA. **This kit does not contain azide- or mercury-based preservatives.**

Summary and Explanation:

Ganz and colleagues discovered hepcidin as an antimicrobial peptide produced in the liver (Park et al., 2001) and, together with other investigators in the field (Pigeon et al., 2001; Nicolas et al., 2001), identified hepcidin as a peptide hormone that regulates extracellular iron in response to changes in dietary and systemic iron load, anemia, hypoxia, erythropoiesis, and inflammation (Ganz, 2005). Hepcidin is an acute phase protein increased in anemia of inflammation. IL-6 is a principal regulator of hepcidin during inflammation (Nemeth et al., 2004). Hepcidin inhibits cellular iron efflux by binding to and inducing degradation of the sole known iron channel, ferroportin. Synthetic hepcidin injected into mice binds to ferroportin-rich tissues and rapidly lowers serum iron levels (Rivera et al., 2005). It is now well established that hepcidin is the master regulator of iron homeostasis in vertebrates.

Data on the normal ranges of circulating levels of the hepcidin-1 protein in common mouse model strains (e.g. C57BL6) are not available currently. Murine hepc-1 protein levels are known to scale closely with dietary iron concentration of the feed provided to mice and to be correlated with hepc-1 gene expression measured by qPCR (Gutschow et al., 2015).

Principle of the Test:

This kit is a solid-phase enzyme-linked immunosorbent assay (ELISA), based on the principles of competitive binding. Murine samples, standards, or controls are mixed with buffer containing hepc-1 biotin conjugate. This mixture is then incubated in an anti-murine hepc-1 antibody coated ELISA microwell plate. The more murine hepc-1 in the sample, the less hepc-1 biotin conjugate will bind to the antibody coated well due to “competition” for antibody binding sites between native hepcidin and hepc-1 biotin conjugate. The plate is then washed to allow the removal of unbound hepc-1 biotin conjugate. The hepc-1 biotin conjugate bound to the antibody is detected with streptavidin conjugated horseradish peroxidase (HRP). The binding of the hepc-1 biotin conjugate is quantified by the addition of TMB, a chromogenic substrate. The reaction produces a blue color and is stopped with the addition of a stop solution and the absorbance is then read at 450 nm. A standard curve is produced by plotting the concentration of the standard versus the absorbance. The intensity of the color is inversely proportional to the concentration of hepcidin-1 in the sample. The total assay run time is 2.75 hours.

Materials and Storage:

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

Materials Provided	1 Kit	SKU#
1. Microwell strip plate coated with anti-Hepcidin-1	12x8x1	-
2. Hepcidin-1 Standard (1 glass vial, clear)	500ng	XMS-500
3. Hepcidin-1 Biotin Conjugate (1 glass vial, amber)	1 vial	XMT-077
4. HRP Conjugate, 1 tube (conc., 100X)	150µl	CEC-001
5. Sample Diluent, 1 bottle (conc., 10X)	4ml	CSD-001
6. Wash Solution, 1 bottle (conc., 25X)	25ml	CWB-001
7. TMB Substrate, 1 bottle (ready to use)	12ml	CTM-001
8. Stop Solution, 1 bottle (ready to use)	12ml	CST-001
9. Microplate sealing film	2	-
10. Polypropylene 96-well Sample Set-up Plate	1	-

Materials Not Provided:

1. Precision pipettes and tips
2. Squirt bottle, manifold dispenser, or automated microplate washer
3. Reagent grade water (deionized or distilled)
4. Microplate reader (450nm), horizontal orbital microplate shaker
5. Tubes to dilute sample diluent, wash solution, hepc-1 conjugate and HRP conjugate

Warnings and Precautions:

Kit does not contain azide- or mercury-based preservatives. For research use only.

1. Use separate pipette tips for each sample, standard, and reagent to avoid cross-contamination.
2. Use separate reservoirs for each reagent, especially the TMB Substrate.
3. The Stop Solution contains 0.5M sulfuric acid. Use appropriate protection.
4. Hemolyzed, hyperlipemic, heat-treated or contaminated samples may give erroneous results.
5. Do not dilute samples directly in the antibody-coated microwell plate.
6. Do not touch or scrape bottom or sides of antibody-coated wells of the microplate.
7. Incubation times and temperatures other than those specified may give erroneous results.
8. Do not allow the wells to dry once the assay has begun.
9. Do not reuse microwell plate or pour reagents back into their bottles once dispensed.
10. The components in this kit are an integral unit. All reagents should be used within 12 hours of preparation. Reagents from different lots should not be mixed. Do not refrigerate or freeze aliquots of any reagent for future use.

Specimen Collection and Handling:

Serum and urine specimens can be used for the assay.

Serum and urine samples are optimal; use of plasma requires further investigation. Collect serum samples according to standard techniques. Centrifuge samples to remove lipids and cellular debris. For long term sample storage, aliquot in small volumes and freeze at -80°C. Urinary hepcidin can be unstable; avoid long term storage of this matrix. Avoid repeated freeze-thaw cycles. Thaw samples and allow them to equilibrate to room temperature for 30 minutes before use; samples must be mixed before analysis.

Sample Dilution:

Dilute samples to 2.5-10% of the recommended 100µl assay volume. It is recommended to begin at 5% sample dilution. Therefore 12µl of sample is required to begin quantification of hepcidin in duplicate using 5% sample dilution (see instructions below).

Preparation of Reagents:

This kit has sufficient reagents and is designed to run an 8-point standard curve and 40 samples in **duplicate only**.

Bring all samples and reagents to room temperature (20-25°C) before use.

1. 1X Wash Solution: Transfer contents of concentrated **Wash Solution** bottle (25ml) to 600ml of reagent grade water.
2. 1X Sample Diluent: Transfer contents of the concentrated **Sample Diluent** bottle (4ml) to 36ml of reagent grade water.
3. 1X HRP Conjugate: Pipette 120µl of the concentrated **HRP Conjugate** into 12ml of the 1X Sample Diluent.
4. Biotin Conjugate: Add 0.5 ml of 1X Sample diluent into Hepcidin-1 Biotin Conjugate vial, mix by vortex then transfer into 15ml tube containing 13.5ml of 1X Sample Diluent. Mix gently by inverting the tube a few times.
5. Biotin Conjugate for Standard: We recommend using a 5% sample dilution. Prepare 4 ml by mixing 0.2 ml of 1X Sample Diluent with 3.8 ml of Biotin Conjugate. To prepare a 2.5% sample dilution for example, combine 0.1ml of Sample Diluent with 3.9 ml of Biotin Conjugate (total 4ml Biotin Conjugate for Standard). To prepare a 10% sample dilution, adjust each reagent in a similar fashion.

Preparation of Standard and Samples:

Standard:

1. Add 0.5 ml Biotin Conjugate for Standard to the **Hepcidin-1 Standard** vial. Mix by pipette.
2. Into the sample set-up plate, transfer 400µl of 1X Hepcidin-1 Standard (well A1) and 250µl Biotin Conjugate for Standard (wells B1-H1). Perform a 125µl serial dilution from well A1 to well G1; leave well H1 undiluted.

Sample:

3. Transfer 228µl of Biotin Conjugate to columns 2 through 6 of the sample set-up plate. Pipette 12µl of sample to achieve a final volume of 240µl/well (5% sample dilution). For a 2.5% sample dilution, add 6µl of sample to 234µl of Biotin Conjugate. To prepare a 10% sample dilution, adjust each reagent in a similar fashion.

Assay Procedure:

1. Remove microwell plate from the foil pouch and wash three times with 1X Wash Solution (300µl/well).
2. Transfer 100µl/well of standard curve and samples from the sample set-up plate to the microwell assay plate in duplicate.
3. Apply sealing film and incubate on an orbital shaker (350 rpm) at room temperature for **2 hours**.
4. Wash microwell plate three times with 1X Wash Solution (300µl/well).
5. Transfer 100µl/well of the 1X HRP Conjugate solution to the microwell plate and incubate for **30 minutes** at room temperature on the orbital shaker.
6. Wash microwell plate three times with 1X Wash Solution (300µl/well).
7. Develop the microwell plate by adding 100µl/well TMB Substrate. Incubate for **15 minutes** at room temperature. Protect from ambient light.
8. Stop the reaction by adding 100µl/well Stop Solution exactly 15 minutes after the addition of the TMB Substrate.
9. Measure absorbance at 450nm of the microwell plate using a plate reader.