Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
info@listarfish.it
www.listarfish.it

Manual

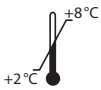
For professional use only

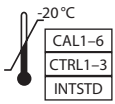
Hepcidin-25 LC-MS/MS kit

For the in vitro determination of hepcidin-25 in plasma and serum

Valid from 2024-01-11

REF KM4000





IVD

CE

Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: +49 6251 70190-0 Fax: +49 6251 70190-363
e.mail: info@immundiagnostik.com www.immundiagnostik.com

Safety information

The assay has to be performed exclusively according to the instructions for use enclosed with the kit. Important safety information for this product can be found in chapter 12.

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1. INTENDED USE

The Hepcidin-25 LC-MS/MS Kit is an *in vitro* diagnostic tool for the quantification of the peptide Hepcidin-25 in plasma and serum by LC-MS/MS after solid phase extraction. The assay is an *in vitro* diagnostic tool for manual and automated use by professional laboratory personnel. It is used to assess iron status by predicting hepcidin-inhibited enteral iron absorption.

2. INTRODUCTION

Hepcidin-25 is a cysteine-rich peptide that is produced in the liver from an 84-amino acid precursor, pre-hepcidin. So far, four isoforms have been characterised which are distinguished by amino-terminal truncations: hepcidin-20, -22, -24 and -25. Hepcidin-20 and -25 are the two main forms consisting of 20 and 25 amino acids, respectively.

Mass spectrometric analysis allows the differentation of these two isoforms and thus the selective determination of hepcidin-25. The natively folded hepcidin-25 contains eight cysteines linked by intramolecular disulfide bridges and regulates, among other things, the absorption of iron into the body.

Binding of hepcidin-25 to ferroportin, the iron export channel, eventually leads to lyosomal degradation of ferroportin. This inhibitory binding prevents the absorption of iron into the bloodstream.

Hepcidin-25 can be used as a predictor of iron absorption. Hepcidin-25 enables the differentation of iron deficiency anaemia (IDA) from anaemia in chronic diseases (ACD) and ACD/IDA. It also predicts the response to oral iron therapy and thus provides valuable information for the treatment of patients.

3. PRINCIPLE OF THE TEST

A simple solid phase extraction (SPE) in the 96-well format is used for hepcidin-25 enrichment. Following chromatographic separation at moderate pressures, the analyte is measured via tandem mass spectrometry (LC-MS/MS).

A stable isotope-labelled version of the analyte is used as internal standard to correct sample loss during the sample preparation and matrix effects during ionisation.

4. MATERIALS PROVIDED

Art. no.	Label	Kit components	Amount
KM0001	ACTSOL	Activation solution	1.5 ml
KM0002	RECSOL	Reconstitution solution	15 ml
KM0003	WASHSOL	Wash solution	80 ml
KM4000	CAL1–6	Calibrators 1–6; lyophilised (see product specification for concentration)	2 vials (á 500 µl) per level each
	CTRL1–3	Control 1–3; lyophilised (see product specification for concentration)	2 vials (á 500 µl) per level each
	DILSOL	Dilution solution	10 ml
	ELUSOL	Elution solution	10 ml
	INTSTD	Internal standard concentrate	3 x 10 µl
	MOPHAA	Mobile phase A	500 ml
	MOPHAB	Mobile phase B	500 ml
	SAMPLEBUF	Sample buffer	30 ml
	WASHSOL2	Wash solution 2	25 ml

For reorders of single components, please use the catalogue number followed by the label without space as product number.

The following accessories for the hepcidin-25 LC-MS/MS kit can be ordered seperately at Immundiagnostik AG:

- tuning solution for hepcidin-25 (KM4000TU)
- tuning solution for the internal standard (KM4000TS)
- HPLC column (KM4000SP)
- precolumn (KM4000VS)
- 96-well solid phase extraction plate (KM400096SPE)
- elution plate (KM40000 PLATE)

Please ask for our single component price list.

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- reaction tubes and pipette tips (see application note)
- Precision pipettors and disposable tips to deliver 10–1 000 µl
- Vacuum station for 96-well solid phase extraction plate
- Vortex-mixer

- LC-MS/MS equipment
- LC-MS vials
- Methanol p.a.
- Ultrapure water*

  * Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

6. PREPARATION, STORAGE AND STABILITY OF REAGENTS

Storage

The test reagents should be stored protected from light, dry and their specified storage temperature (CAL1–6, CTRL1–3, INTSTD: -20 °C; all others 2–8 °C). The test reagents stored in this way are usable until the indicated expiry date.

Attention: The -20 °C components should not be refrozen after reconstitution / dilution.

Note: After preparation of the reagents their stability may vary (see respective preparation step).

Preparation of mobile phases and test reagents

Before use, the mobile phases (MOPHAA and MOPHAB), sample buffer (SAMPLEBUF), wash solution 2 (KM4000WASHSOL2), dilution solution (DILSOL) and elution solution (ELUSOL) must be activated by adding activation solution (ACTSOL) according to the following chart:

Component			ACTSOL [µl]
Name	[ml]		
Mobile phase (MOPHAA and MOPHAB)	500		500
Sample buffer (SAMPLEBUF)	30		60
Washing solution 2 (WASHSOL2)	25	+	25
Dilution solution (DILSOL)	10		10
Elution solution (ELUSOL)	10		10

Prior use mobile phases should be degassed.

Note: After activation with activation solution (ACTSOL), the components mobile phase A (MOPHAA), mobile phase B (MOPHAB), sample buffer (SAMPLEBUF), wash solution 2 (WASHSOL2), dilution solution (DILSOL) and elution solution (ELUSOL)

can be stored at 2–8 °C up to 2 weeks. It is therefore recommended to prepare only as much as is needed for the test approach.

Attention: The activation solution (ACTSOL) must be added under the fume cupboard. All vessels to be used must be absolutely clean, free of detergents and preferably made of LC-MS/MS suitable glass.

Preparation of calibrators, controls and internal standard

Dissolve calibrators (CAL1–6) and controls (CTRL1–3) in 500 µl of reconstitution solution (RECSOL) each while 30 s vortexing.

Note: The reconstituted calibrators (CAL1–6) and controls (CTRL1–3) can be stored at 2–8 °C for up to 1 week.

The internal standard (INTSTD) is diluted in two steps with sample buffer (SAMPLEBUF) immediately before use. Here, necessarily use the pipette tips and reaction vessels recommended in the application note.

Dilution I

Add 190 µl SAMPLEBUF to 10 µl INTSTD (IS dilution I, 1:20). This dilution must be prepared in the INTSTD vial.

Note: IS dilution I can be stored at 2–8 °C for up to 1 week.

If more than one vial of INTSTD is needed for a work-up of several samples and consequently more than one IS-dilution I is prepared, the required volume should first be combined in a suitable reaction tube before preparing IS-dilution II.

Dilution II

Depending on the number of samples to be processed, prepare an appropriate volume of the ready-to-use IS-dilution II, e.g. for 30 samples:

150 µl IS dilution I + 5 850 µl SAMPLEBUF (IS dilution II, 1:40).

Note: IS dilution II must be used immediately after preparation and is not stable.

7. SAMPLE PREPARATION

Serum, citrate, and heparin plasma samples are suited for the assay.

Attention: Interferences may occur with hemolytic samples.

The quality controls should be analysed with each run.

Only reagents and samples that are at room temperature (18–26 °C) shall be used in the test.

Mix well samples and reagents before use.

Note: Reagents used in the SPE step contain organic solvents. When working with these reagents, the legal safety precautions must be observed.

1.	Conditioning of the 96-well solid phase extraction plate (96SPE) with 200 µl methanol per well, aspirate via the vacuum station.
2.	Equilibration with 200 µl ultrapure water, aspirate.
3.	Pipette 200 µl sample, calibrator (CAL1–6) or control (CTRL1–3) into each well.
4.	Add 200 µl internal standard (IS dilution II), aspirate.
5.	First wash step with 200 µl wash solution (KM0003 WASHSOL), aspirate
6.	Second wash step with 200 µl wash solution 2 (activated ASHSOL2), aspirate.
7.	Third wash step with 200 µl wash solution (KM0003 WASHSOL), aspirate.
8.	Change waste container to collection plate before the elution steps.
9.	First elution step with 25 µl elution solution (activated ELUSOL), aspirate.
10.	First elution step with 25 µl elution solution (activated ELUSOL), aspirate.
11.	Dilution (1:2) of the combined elution fractions with dilution solution (activated DILSOL): e. g. 50 µl eluate + 50 µl dilution solution This step can be performed direct in the elution plate or also after transfer of the eluate to LC-MS vials.
12.	Injection into the LC-MS/MS system (see application note).

8. LC-MS/MS METHOD

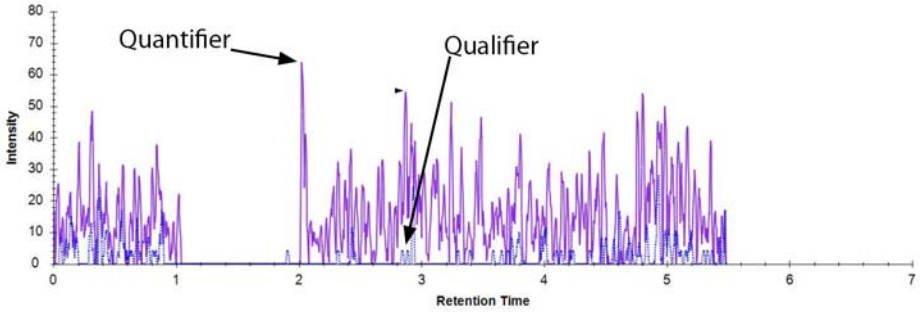
Please refer to the application note or contact lcms@immundiagnostik.com for the parameters for setting the LC-MS/MS method.

9. EXAMPLES OF CHROMATOGRAMS

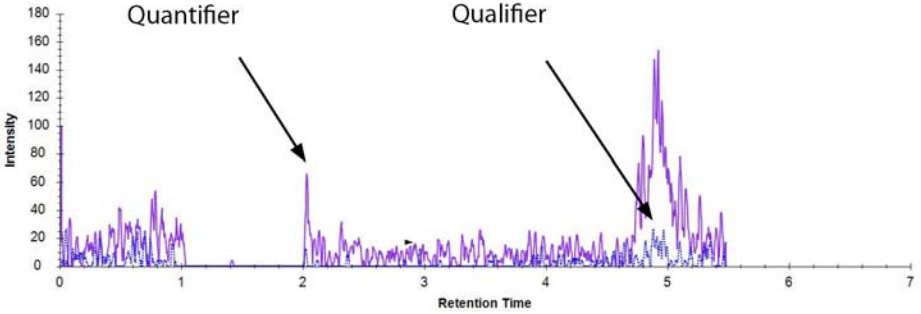
It must be noted that the retention time and signal intensity may vary depending on the device.

Blank

Analyte transitions

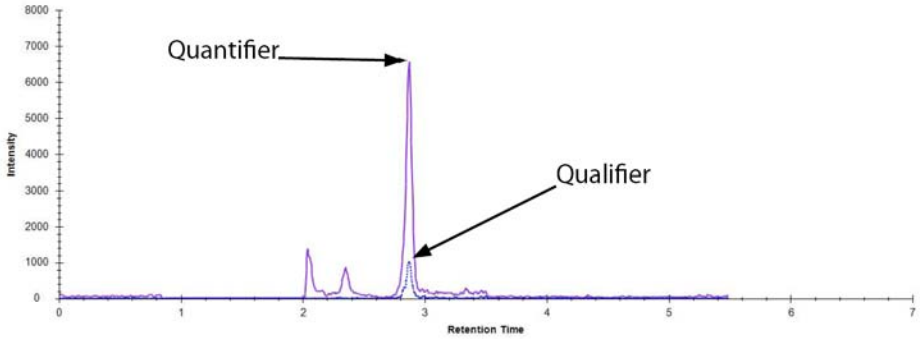


Internal standard transitions

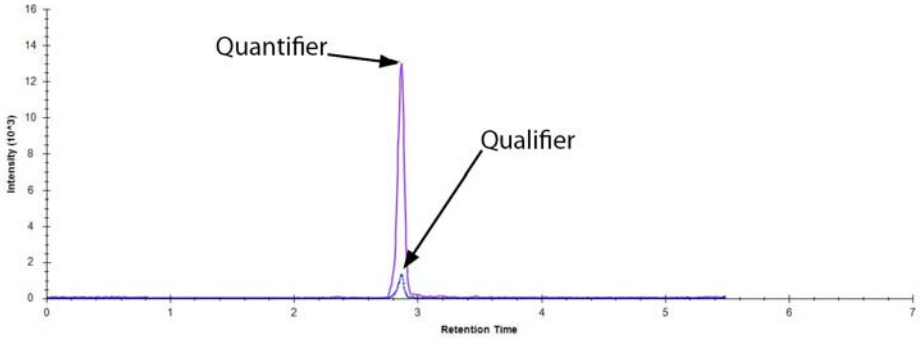


Control 1 (CTRL1)

Analyte transitions

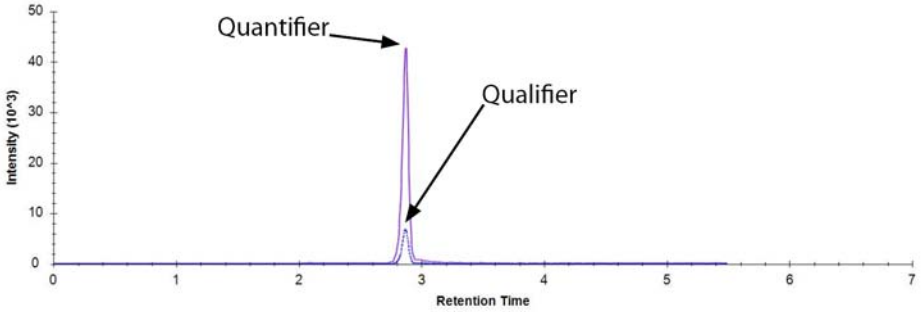


Internal standard transitions

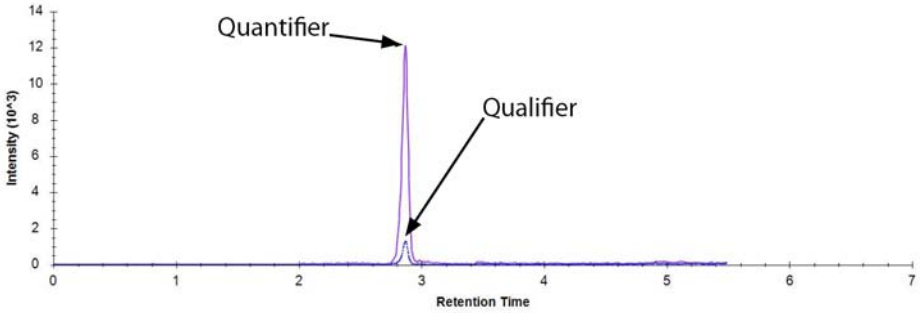


Sample

Analyte transitions



Internal standard transitions



10. QUALITY CONTROL

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits (see product specification).

Reference range

Preliminary reference range (serum and plasma), Galesloot *et al.*, 2011

Men, n = 1 066: 1.39–43.24 ng/ml (P2.5–P97.5), Median: 13.11 ng/ml

Women, n = 882: 1.39–42.96 ng/ml (P2.5–97.5), Median: 10.60 ng/ml

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Accuracy and precision

	Accuracy		**Precision**	
sample [ng/ml]	***intra-day* (n=5)**	***inter-day* (n=15)**	***intra-day* (n=5)**	***inter-day* (n=15)**
1.9	93.5 %	98.0 %	6.8 %	6.8 %
5.3	106.5 %	103.3 %	6.2 %	5.4 %
19.9	104.0 %	100.2 %	3.8 %	3.8 %
92.5	107.4 %	102.5 %	5.3 %	5.2 %

Sensitivity / Limit of quantification (LLOQ)

The LLOQ designates the lowest concentration of the analyte that can still be quantified:

hepcidin-25: 1.9 ng/ml

It is important to note that the quantification limit is not exclusively application-dependent, but also device-dependent.

12. PRECAUTIONS

- Control samples should be analysed with each run.
- Human material used in the kit components was tested and found to be negative for HIV, hepatitis B and hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- The GHS symbols indicated on the individual components and specifications of the material safety data sheets (available on request from Immundiagnostik AG) must be noted. When working with these reagents, the legal protective precautions must be adhered to.
- As a precaution, it is recommended that the human material used is always considered potentially infectious.

13. DISPOSAL

- Mobile phases (MOPHAA and MOPHAB), sample buffer (SAMPLEBUF), wash solution 2 (KM4000WASHSOL2), elution solution (ELUSOL), dilution solution (DILSOL) and activation solution (ACTSOL) must be deposited as non-halogenated solvents. The calibrators (CAL1–6) and controls (CTRL1–3) should be disposed due to their treatment as potentially infectious material in accordance with local regulations.

14. TECHNICAL HINTS

- Do not mix different lot numbers of any kit component.
- Reagents should not be used beyond the expiration date stated on the kit label.
- The assay should always be performed according to the enclosed manual.
- Plugs and caps of different reagents should not be swapped.
- The individual components of the kit are designed for a maximum of the specified number of test runs. Any part of the components that has already been used must not be reused, but must be disposed of properly in accordance with local regulations.

15. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- The guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- All serious incidents occurring in connection with the product must be reported to Immundiagnostik AG and (within the Union market) to the competent reporting authority of the respective member state.
- Please contact Immundiagnostik AG if one or more components of the kit are damaged, missing (see material supplied) or precipitates are visible in the ready-to-use solutions.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

16. REFERENCES

1. Bregman DB, Morris D, Koch TA, He A, Goodnough LT. Hepcidin levels predict nonresponsivenessto oral iron therapy in patients with iron deficiency anemia. *Am J Hematol*. 2013;**88**(2):97-101.
2. Galesloot TE, Vermeulen SH, Geurts-Moespot AJ, et al. Serum hepcidin: reference ranges and biochemical correlates in the general population. *Blood*. 2011;**117**(25):e218-e225.
3. Hershko C, Camaschella C. How I treat unexplained refractory iron deficiency anemia. *Blood*. 2014;**123**(3):326-333.
4. Rochette L, Gudjoncik A, Guenancia C, Zeller M, Cottin Y, Vergely C. The iron-regulatory hormone hepcidin: a possible therapeutic target?. *Pharmacol Ther*. 2015;**146**:35-52.

17. SYMBOL EXPLANATION


Temperature limitation

In Vitro Diagnostic Medical Device


Manufacturer

Lot number


Contains plasma derivatives or human blood

Consult specification data sheet

Unique Device Identification


Medicinal substance

Catalogue number

To be used with

Contains sufficient for <n> tests


Use by


Consult instructions for use


Do not re-use

Contains material of animal origin

Contains material of human origin