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**Manual**

# *IDK*® Casomorphin/Gliadorphin peptides LC-MS/MS

***For the determination in stabilised urine***

Valid from 2021-10-04

REF **KM8000**

Σ 100

+8°C +2°C

-20°C CAL CTRL

IVD CE

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# Table of Contents

## 1. INTENDED USE

The *IDK*® Casomorphin/Gliadorphin peptides LC-MS/MS kit is an *in vitro* diagnostic tool for the quantitative determination of casomorphin/gliadorphin peptides in stabilised urine by LC-MS/MS after solid phase extraction. The kit is intended for manual use by professional laboratory staff.

## 2. INTRODUCTION

Exorphins are bioactive peptides which can react with opiate receptors. These peptides are derived from e. g. the incomplete hydrolysis of the milk protein casein and the wheat protein gluten and gliadin. Casomorphin (BCM7 from casein) and gliadorphin-7 (from gluten) have been widely studied and are linked to gastrointestinal, neurological, and neuro-developmental disorders.

These short peptides are usually broken down by the enzyme dipeptidyl-peptidase IV (DPPIV). In people with autism, schizophrenia and other neurological disorders, either a genetic deficiency or inhibition of this enzyme, e.g. by drugs treating diabetes type 2, mercury or yeast, can occur.

Both ways, this proteolytic enzyme can't act properly and leaves incompletely digested peptides behind which are able to pass the blood-brain barrier after the uptake from the intestinal lining into the bloodstream. In the brain, these bioactive peptides can bind to opiate receptors and mimic the effects of opiate drugs like heroine and morphine. This can result in change of behavior, lack of focus and attention, sleepiness and even aggression and self-abuse.

Wheat and milk allergies are associated casomorphin/gliadorphin problems. Even if no milk or wheat allergies occur some people can still react negatively to these peptides.

Children with autism and ADHS can benefit from an elimination diet – gluten-free, casein-free (GfCf) diet. Physical and mental health can be improved.

This test for casomorphin/gliadorphin peptides is superior to any other tests because no nutrional regimen, e. g. abandonment of soy products, prior to sample collection is required.

## 3. MATERIAL SUPPLIED

| Art. no. | Label | Kit components | Amount |
|---|---|---|---|
| 2 x KM0001 | 2 x ACTSOL | 2 x Activation solution | 2 x 1.5 ml |
| 4 x KM0002 | 4 x RECSOL | 4 x Reconstitution solution | 4 x 15 ml |
| 6 x KM0003 | 6 x WASHSOL | 6 x Wash solution | 6 x 80 ml |
| KM8000 | CAL1–2 | Calibrators 1 und 2, lyophilised (for concentration see product specification) | 5 vials (á 2.2 ml) per level each |
| | CTRL1–2 | Controls 1 und 2, lyophilised (for concentration see product specification) | 5 vials (á 2.2 ml) per level each |
| | ELUSOL | Elution solution | 2 x 27 ml |
| | INTSTD | Internal standard | 1.5 ml |
| | MOPHAA | Mobile phase A | 1 000 ml |
| | MOPHAB | Mobile phase B | 1 000 ml |
| | SOLA | Solution A | 22 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 *IDK*® Casomorphin/Gliadorphin peptides LC-MS/MS kit can be ordered seperately at Immundiagnostik AG:

- tuning solution for the analytes (KM8000TU)
- tuning solution for the internal standard (KM8000TS)
- UPLC column (KM8000SP)
- in-line filter (KM8000IF)
- in-line filter holder (KM8000IH)
- SPE cartridge (KM8000CK)

Please ask for our single component price list.

## 4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Standard laboratory glass vials, suitable for LC-MS/MS
- Calibrated precision pipettors and 10–1 000 µl tips
- Solid phase extraction unit
- Evaporation unit (e. g. vacuum centrifuge or nitrogen distributor)
- Vortex
- LC-MS/MS equipment
- Borosilicate tubes

- Methanol p. a.
- Ultra pure water*

  * Immundiagnostik AG recommends the use of Ultra Pure 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).

## 5. STORAGE AND PREPARATION OF REAGENTS

### *Storage*

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

**Note:** After preparation of the test reagents for the test procedure other stabilities might apply (see respective preparation step).

### *Preparation of mobile phases and test reagents*

Before use, the mobile phases (MOPHAA and MOPHAB) and solution A (SOLA) must be activated by adding activation solution (ACTSOL) according to the following chart:

| Component | | | ACTSOL [µl] |
|---|---|---|---|
| Name | [ml] | | |
| Mobile phase A (MOPHAA) | 500 | | 500 |
| Mobile phase B (MOPHAB) | 500 | + | 500 |
| Solution A (SOLA) | 25 | | 25 |

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.

### *Preaparation of the calibrators and controls*

The calibrators (CAL1–2) and the controls (CTRL1–2) are dissolved in 2.2 ml reconstitution solution (RECSOL) each while 30 s vortexing.

**Note:** After reconstitution with the reconstitution solution (RECSOL), the calibrators (CAL1–2) and the controls (CTRL1–2) are not stable and can not be stored.

## 6. STORAGE OF SAMPLES

In unstabilised urine, the concentration of casomorphin/gliadorphin peptides declines quickly, therefore only stabilised urine is suited as sample. Immundiagnostik offers urine sample stabilising tubes (order no DZ9030UT) specifically designed for this purpose. Samples stabilised using those tubes are stable for 48 h at room temperature. For longer storage, the sample must be frozen at < -17 °C.

## 7. ASSAY PROCEDURE

Control samples should be analysed with each run.

Prior to use in the assay, allow all samples and reagents to come to room temperature (18–26 °C).

Mix samples and reagents well before use.

### *Preparation of the cartridges*

Conditioning of the cartridges with 1 ml methanol, followed by equilibration with 1 ml ultra pure water.

### *Test procedure*

| | |
|---|---|
| 1. | Mix 1 ml reconstituted CAL/reconstituted CTRL/sample and 10 µl INTSTD in a reaction tube. |
| 2. | Add solution onto the SPE cartridges and aspirate. |
| 3. | Wash with 1 ml wash solution (WASHSOL), aspirate. Carry out this wash step four times in total. |
| 4. | Eluate with 500 µl elution solution (ELUSOL) into borosilicate tubes. |
| 5. | Dry the eluate, e. g. in a vacuum centrifuge. |
| 6. | Reconstitute the dried sample with 200 µl activated solution A (SOLA). |
| 7. | Injection into the LC-MS/MS system (see applicatio note). |

## 8. LC-MS/MS METHOD

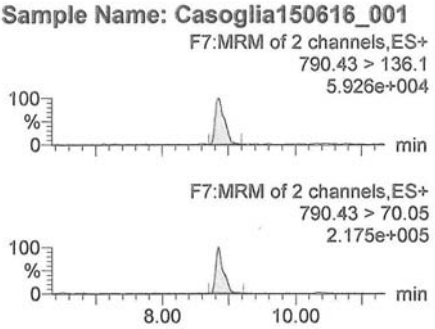
Please refer to the application note or contact lcms@immundiagnostik.com for the parameters for setting the LC-MS/MS method.

## 9. CALCULATION

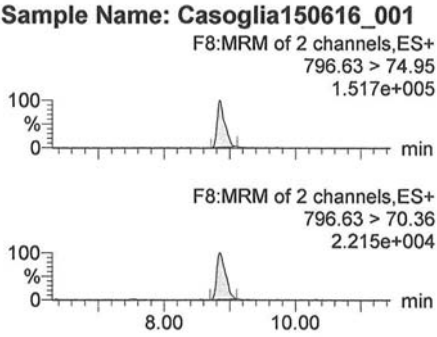
The linear regression is used as model for evaluation of the results. The two calibrator concentration points are connected by a strait line. The samples can be calculated using the obtained line.

## 10. CHROMATOGRAM EXAMPLES

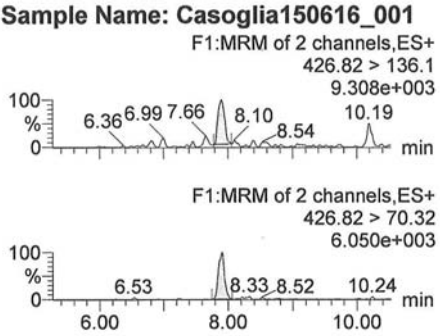
**β-Casomorphin 7** (molecular weight: 789.95 g/mol)



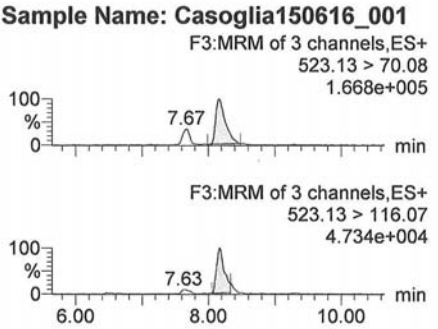

**INTSTD** (β-Casomorphin 7 heavy labelled, molecular weight: 795.95 g/mol)



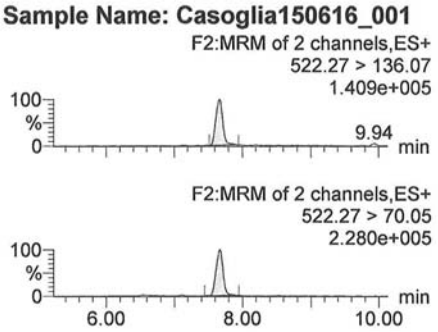
**Casomorphin 1–3** (molecular weight: 425.50 g/mol)



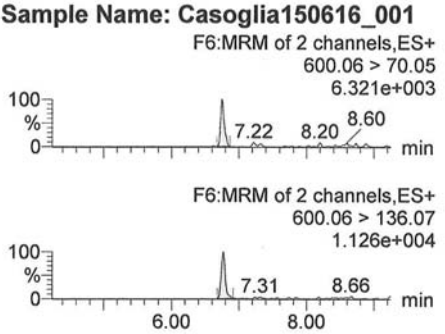

**Casomorphin 1–4** (molecular weight: 522.62 g/mol)



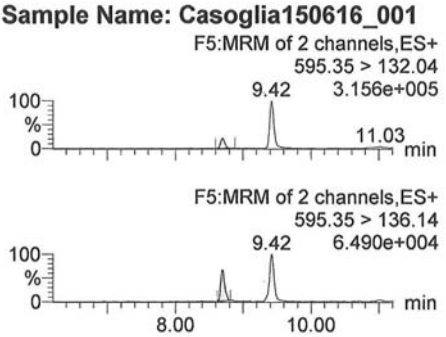

**Casomorphin 1–4 amide** (molecular weight: 521.63 g/mol)



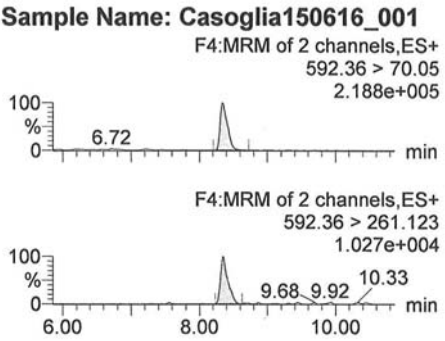
**Exorphin A5** (molecular weight: 599.65 g/mol)



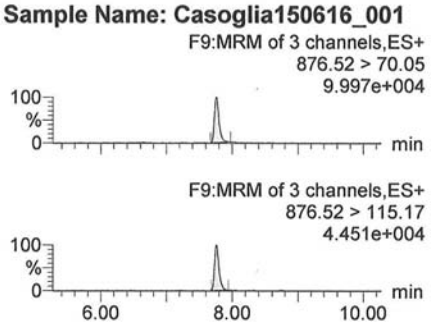

**Exorphin B5** (molecular weight: 594.67 g/mol)




**Exorphin C** (molecular weight: 591.72 g/mol)



**Gliadorphin** (molecular weight: 876.00 g/mol)




## 11. QUALITY CONTROL

Control samples should be analysed 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.

### *Reference range*

Based on Immundiagnostik studies of samples of apparently healthy persons (n = 18), the following mean values were estimated

| Analyte | ng/µmol Creatinine |
|---|---|
| Casomorphin 1–3 | 1.406 |
| Exorphin A5 | 2.033 |
| ß-Casomorphin 7 | 0.138 |
| Gliadorphin | 0.187 |
| Casomorphin 1–4 amide | 0.234 |
| Casomorphin 1–4 | 0.188 |
| Exorphin C | 0.076 |
| Exorphin B5 | 0.611 |

We recommend each laboratory to establish its own reference range.

## 12. PERFORMANCE CHARACTERISTICS

*Precision - reproducibility and repeatability*

**Inter-Assay (n = 10)**

| Analyte | Sample | ng/ml | CV [%] |
|---|---|---|---|
| Casomorphin 1–3 | 1 | 1.05 | 10.79 |
| | 2 | 4.55 | 2.41 |
| Exorphin A | 1 | 3.75 | 24.35 |
| | 2 | 11.37 | 9.27 |
| ß-Casomorphin 7 | 1 | 1.56 | 12.32 |
| | 2 | 6.93 | 4.66 |
| Gliadorphin | 1 | 1.96 | 7.38 |
| | 2 | 7.80 | 7.14 |
| Casomorphin 1–4 amide | 1 | 1.44 | 11.40 |
| | 2 | 5.57 | 6.07 |
| Casomorphin 1–4 | 1 | 2.09 | 5.13 |
| | 2 | 8.71 | 5.54 |
| Exorphin C | 1 | 2.84 | 5.16 |
| | 2 | 11.76 | 8.53 |
| Exorphin B | 1 | 2.88 | 5.37 |
| | 2 | 10.91 | 4.31 |

**Intra-Assay (n = 4)**

| Analyte | Sample | ng/ml | CV [%] |
|---|---|---|---|
| Casomorphin 1–3 | 1 | 3.44 | 12.92 |
| | 2 | 1.14 | 3.63 |
| Exorphin A | 1 | 6.29 | 26.98 |
| | 2 | 3.05 | 39.92 |
| ß-Casomorphin 7 | 1 | 3.19 | 8.42 |
| | 2 | 1.52 | 11.88 |

| Analyte | Sample | ng/ml | CV [%] |
|---|---|---|---|
| Gliadorphin | 1 | 3.17 | 8.03 |
| | 2 | 2.14 | 7.59 |
| Casomorphin 1–4 amide | 1 | 2.33 | 17.08 |
| | 2 | 1.34 | 15.58 |
| Casomorphin 1–4 | 1 | 2.83 | 7.79 |
| | 2 | 2.06 | 7.77 |
| Exorphin C | 1 | 3.91 | 4.11 |
| | 2 | 3.05 | 4.69 |
| Exorphin B | 1 | 5.24 | 13.38 |
| | 2 | 2.72 | 8.84 |

*Sensitivity*

The detection limit (LLOD) designates the lowest concentration of the analyte that can still be detected.

| Analyte | pg/ml Urine |
|---|---|
| Casomorphin 1–3 | 1 132 |
| Exorphin A | 1 872 |
| ß-Casomorphin 7 | 420 |
| Gliadorphin | 520 |
| Casomorphin 1–4 amide | 624 |
| Casomorphin 1–4 | 307 |
| Exorphin C | 473 |
| Exorphin B | 976 |

It should be noted that the detection limit depends not only on the application method but also on the instrument.

## 13. PRECAUTIONS

- Control samples should be analysed with each run.
- Human materials used in kit components were 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.

## 14. DISPOSAL

Mobile phase B (MOPHAB), elution solution (ELUSOL), solution A (SOLA) and activation solution (ACTSOL) must be disposed as non-halogenated solvents. The calibrators (CAL1–2) and controls (CTRL1–2) should be disposed due to their treatment as potentially infectious material in accordance with local regulations.

## 15. TECHNICAL HINTS

- Do not mix different lot numbers of any kit component.
- Reagents should not be used beyond the expiration date shown 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.

## 16. 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.
- *IDK*® is a trademark of Immundiagnostik AG.

- 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.
- 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.

***Used symbols:***