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Manual

Pregnenolone sulfate LC-MS/MS kit

For the determination of pregnenolone sulfate in serum and plasma

Valid from 2021-10-21

REF **KM2000**

Σ 100

+8 °C
+2 °C

-20 °C
CAL1-6
CTRL1-3
PREC

IVD

CE

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1. INTENDED USE

The pregnenolone sulfate LC-MS/MS kit is an *in vitro* diagnostic tool for the quantitative determination of pregnenolone sulfate by LC-MS/MS in serum and plasma after precipitation reaction. The kit is for manual and automatic use by professional laboratory staff. As a precursor for the biosynthesis of neurosteroid hormones, the quantification of pregnenolone sulfate can contribute to the monitoring of these physiological parameters.

2. INTRODUCTION

Steroids and neurosteroids are synthesized from cholesterol, the basic building block of all steroid hormones. While the classic steroids are produced in tissues like adrenal gland, gonads or placenta, the synthesis of neurosteroids takes place in the central and peripheral nervous system. In the 1980s, Baulieu et al. demonstrated that several steroids, such as dehydroepiandrosterone and pregnenolone, are synthesized *de novo* in the central nervous system and present in higher concentrations in the brain than in the blood. These steroids are universally referred as neurosteroids and are differently distributed in the brain and the pituitary gland.

Neurosteroids are formed from cholesterol via pregnenolone and progesterone (see Fig.1). Pregnenolone and progesterone are precursors not only of the synthesis of gluco- and mineral corticoids in the adrenal gland and of the sexual hormones in the gonads or placenta, but also of neurosteroids in the central nervous system, i. e. pregnenolone is an endogenous, naturally produced steroid that is a precursor of many body hormones.

Numerous studies have demonstrated that neurosteroids interact with neurotransmitter receptors, influence the brain excitability, thereby functioning as potent allosteric modulators of several neurotransmitter receptors: the endogenous neurosteroid allopregnanolone, a positive modulator of the γ-aminobutyric acid type A ($GABA_A$) receptor complex, activates the $GABA_A$ receptor functions lowered by various stresses, and has anxiolytic and anti-stress effects. In contrast, negative modulators, like dehydroepiandrosterone sulfate and pregnenolone sulfate, inhibit almost completely the $GABA_A$-receptor.

Due to their lipophility, most of the free steroids can easily pass the blood-brain barrier. A number of steroids are conjugated as sulfate or fatty acid esters and occur in concentrations much higher than these of the free steroids. In contrast to the free steroids, they can not pass through the blood-brain barrier and represent the active forms involved in the control of various physiological and pathophysiological processes.

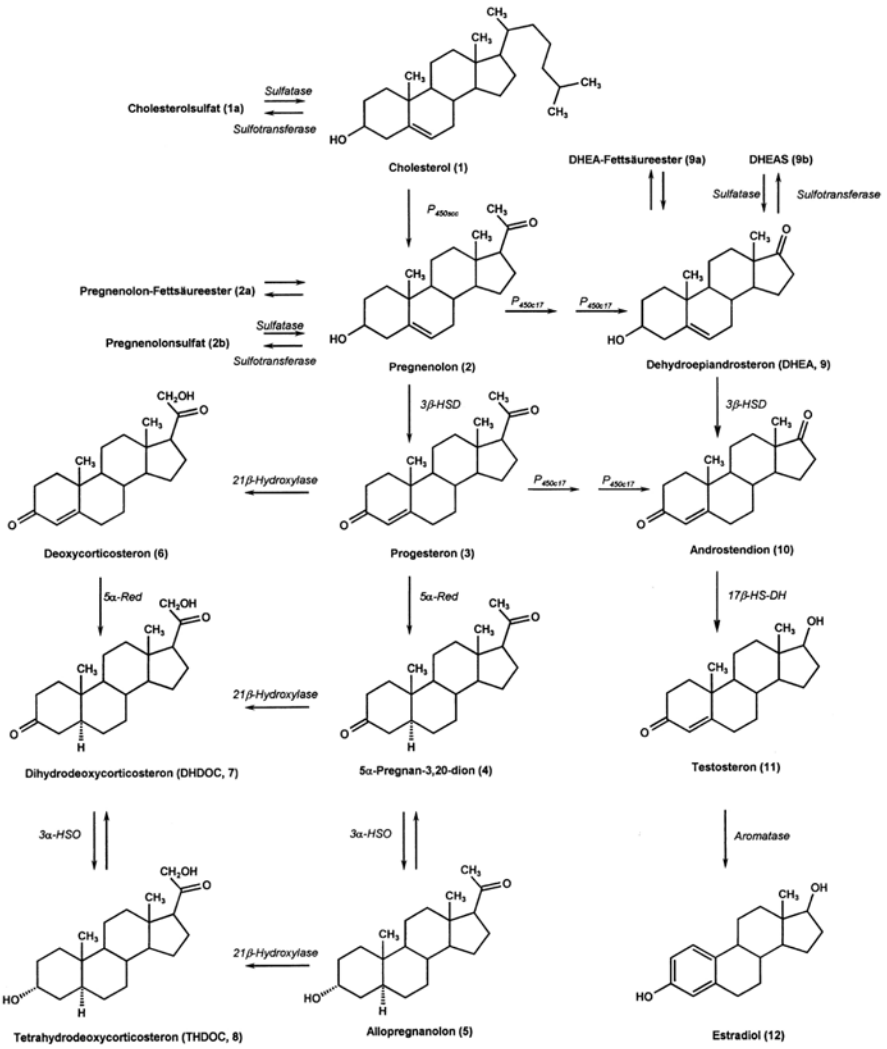


Fig.1: Biosynthesis of neuroactive steroids, Weiss M & Hess S, 2000

Neurosteroids affect a number of processes in the central nervous system, which have a powerful effect on our thinking and feeling. Furthermore, neurosteroids influence the social and sexual behavior. At the same time, they are promising pharmaceutical targets for important indications like epilepsy, anxiety disorders and dementia.

For these reasons, the determination of neurosteroids, especially of pregnenolone and pregnenilone sulfate, is expected to be helpful for understanding the roles of neurosteroids, for diagnosis of psychic or mental disorders and for the development of new steroidal therapeutic agents.

Indications

- Cognitive ability
- Weakness of memory
- Mood swings like depression

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KM0002	RECSOL	Reconstitution solution	15 ml
KM2000	CAL1–6	Calibrators 1-6, lyophilized (see product specification for concentration)	1 vial (à 250 µl) per level
	CTRL1–3	Control 1-3, lyophilized (see product specification for concentration)	1 vial (à 250 µl) per level
	DILSOL	Dilution solution (see sample preparation)	50 ml
	MOPHAA	Mobile phase A	500 ml
	MOPHAB	Mobile phase B	500 ml
	PREC	Precipitation reagent (-20 °C, contains internal standard)	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 pregnenolone sulfate LC-MS/MS kit can be ordered seperately at Immundiagnostik AG:

- tuning solution for pregnenolone sulfate (KM2000TU)
- tuning solution for the internal standard (KM2000TS)
- UPLC column (KM2000SP)
- in-line filter (KM2000IF)
- in-line filter holder (KM2000IH)
- 96-deep well plate (KM2000DP)

Please ask for our single component price list.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- 1.5 ml Eppendorf reaction tubes or 96-deep well plate with fastening option
- Precision pipettors and disposable tips to deliver 10–1 000 µl
- Centrifuge, 8 900 *g* for 1.5 ml Eppendorf reaction tubes or 150 *g* for microtiter plates
- Vortex or microtiter plate mixer
- LC-MS/MS equipment
- LC-MS vials

5. PREPARATION AND STORAGE OF REAGENTS

Storage

The test reagents should be stored protected from light, dry and their specified storage temperature (CAL1–6, CTRL1–3, PREC: -20 °C; all other components 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).

Preaparation of the calibrators and controls

The calibrators (CAL1–6) and the controls (CTRL1–3) are dissolved in 250 µl reconstitution solution (RECSOL) each while 30 s vortexing.

Note: After reconstitution with the reconstitution solution (RECSOL), the calibrators (CAL1–6) and the controls (CTRL1–3) are stable at 2–8 °C for 10 days.

6. SAMPLE PREPARATION

Serum and plasma samples are suited for the assay.

When using citrate plasma, the dilution factor in the blood collection tube with the citrate solution (usually a factor of 1:10) must be taken into account for the later evaluation.

With exception of the precipitation reagent (PREC), only reagents and samples at room temperature (15–30 °C) should be used in the test.

Before use, mix reagents and samples well.

Control samples should be analyzed with each run.

	96-deep well plate	1.5 ml reaction tube
1.	Add 50 µl sample, calibrator (CAL) or control (CTRL) in one well respectively reaction tube.	
2.	Add 250 µl ice-cold (-20 °C) precipitation reagent (PREC).	
3.	Seal plate and shake for 5 min at 1 000 rpm.	Seal tubes and vortex for 1 min.
4.	Centrifuge for 15 min at 150 *g*.	Centrifuge for 15 min at 8 900 *g*.
5.	Add 400 µl dilution solution (DILSOL) in a new well or LC-MS vial.	
6.	Transfer 100 µl of the supernatant (from step 4.) to the DILSOL presented.	
7.	Mix the sample by pipetting up and down.	-
8.	Seal plate and shake for 5 min at 1 000 rpm.	Seal LC-MS vials and vortex for 5 s.
9.	Injection into the LC-MS system (see application note).	

7. LC-MS/MS METHOD

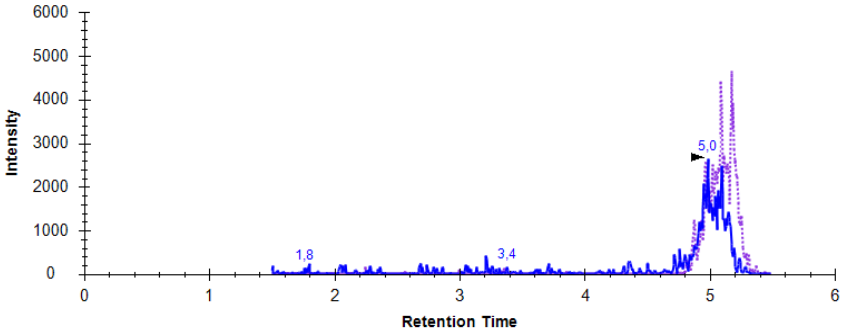
Please refer to the application note or contact lcms@immundiagnostik.com for the parameters for setting the LC-MS/MS method.

8. EXAMPLES OF CHROMATOGRAMS

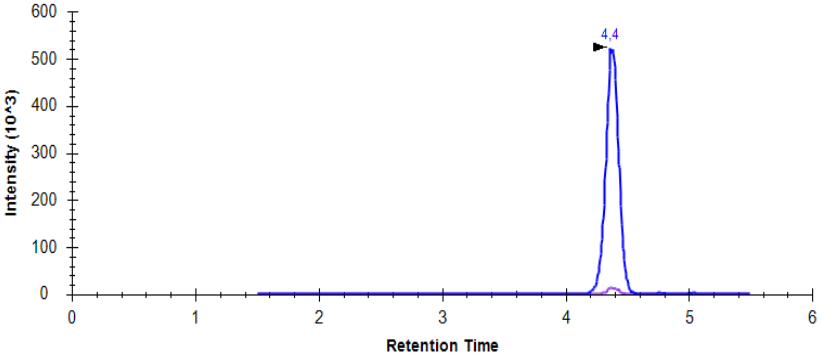
It must be noted that the retention time and signal intensity may vary depending on the device.

Blank

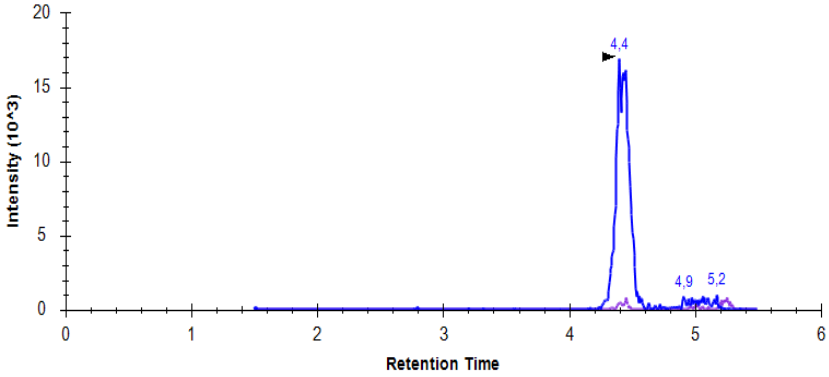
Pregnenolone sulfate



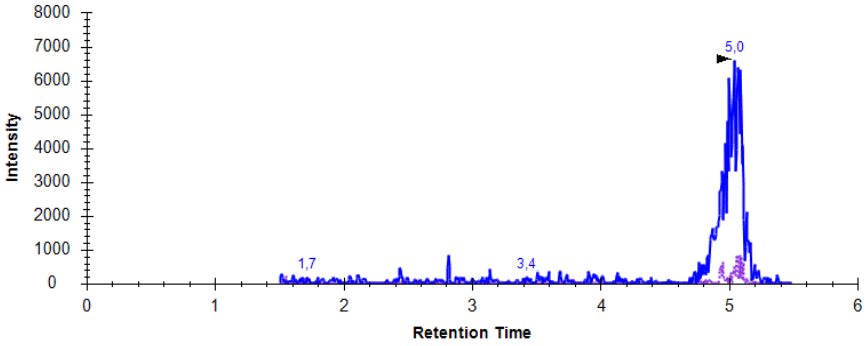
Internal standard



Calibrator pregnenolone sulfate (CAL1)

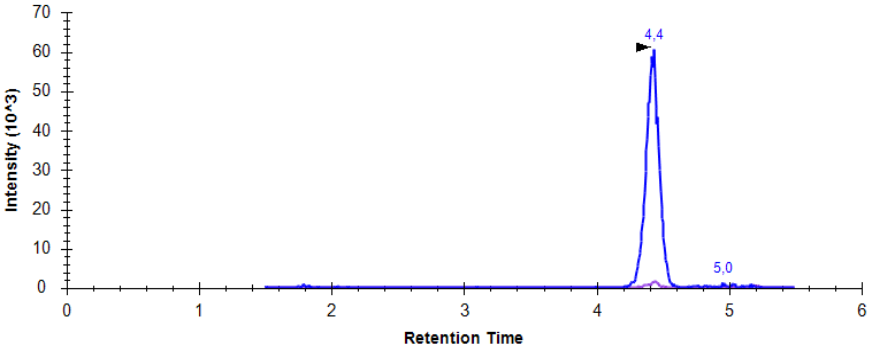


Internal standard

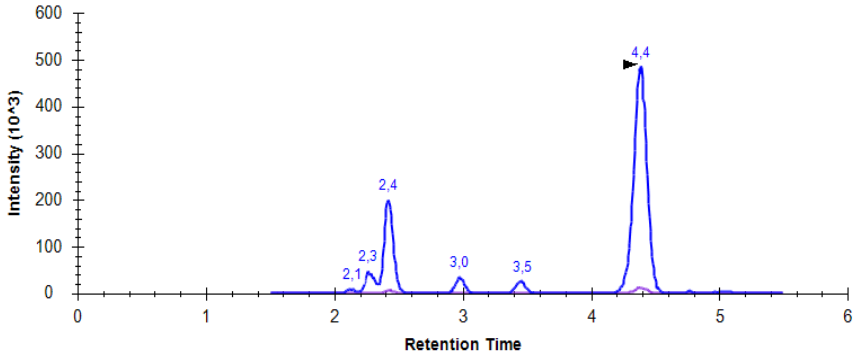


Sample

Pregnenolone sulfate



Internal standard



9. 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

20–155 ng/ml

We recommend each laboratory to establish its own reference range.

10. PERFORMANCE CHARACTERISTICS

Accuracy and precision

96-deep well plate

	accuracy		precision	
sample [ng/ml]	**intra-day (n=5)**	**inter-day (n=15)**	**intra-day (n=5)**	**inter-day (n=15)**
12.2	107.8 %	103.7 %	6.4 %	5.7 %
72.6	106.4 %	103.4 %	5.1 %	3.8 %
145.2	105.5 %	100.3 %	4.5 %	5.1 %

1.5 ml reaction tube

	accuracy		precision	
sample [ng/ml]	**intra-day (n=5)**	**inter-day (n=15)**	**intra-day (n=5)**	**inter-day (n=15)**
12.2	98.8 %	98.1 %	3.3 %	2.3 %
72.6	102.2 %	101.2 %	4.5 %	2.9 %
145.2	101.6 %	100.2 %	2.1 %	1.8 %

Sensitivity / limit of quantification (LLOQ)

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

pregnenolone sulfate: 11.5 ng/ml

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

11. PRECAUTIONS

- The quality control guidelines should be followed.
- 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.

12. 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.

13. DISPOSAL

Mobile phases (MOPHAA, MOPHAB) dilution solution (DILSOL) and precipitation reagent (PREC) must be disposed 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. 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 wrong 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 in respect of deficiencies must be lodged within 14 days after receipt of the product. The product shall be send to Immundiagnostik AG together with a written complaint.

15. REFERENCES

1. Hu, Z Y, Bourreau, E, Jung-Testas, I, Robel, P, Baulieu, E E: Neurosteroids: oligodendrocyte mitochondria convert cholesterol to pregnenolone, *Proc Natl Acad Sci USA* **84** (1987), 8215-8219
2. Rupprecht, R, Holsboer, F: Neuroactive steroids: mechanism of action and neuropsychological perspectives, *Trends Neurosci* **22** (1999), 410-416
3. Mehta, A K, Ticku, M K: An update on GABAA receptors, *Brain Res Brain Res Rev* **29** (1999), 196-217
4. Lambert, J J, Belelli, D, Hill-Venning, C, Peters,J A: Neurosteroids and GABAA receptor function, *Trends Pharmacol Sci* **16** (1995), 295-303
5. Park-Chung, M, Malayev, A, Purdy, R H, Gibbs, T T, Farb, D H: Sulfated and unsulfated steroids modulate γ-aminobutyric acidA receptor function through distinct sites, *Brain Res* **830** (1999), 72-87
6. Monaghan, E P, Navalta, L A, Shum, L, Ashbrook, D W, Lee, D A: Initial human experience with ganaxolone, a neuroactive steroid with antiepileptic activity, *Epilepsia* **38** (1997), 1026-1031

7. Weiss M & Hess S: Neuroaktive Steroide: Wirkungen und Risiken, *Pharmazie in unserer Zeit* **29. Jahrg** (2000) 6: 372-376

8. Jäntti SE, Tammimäki A, Raattamaa H, Piepponen P, Kostiainen R, Ketola RA: Determination of steroids and their intact glucuronide conjugates in mouse brain by capillary liquid chromatography-tandem mass spectrometry. *Anal Chem*. **82** (2010) Apr 15; (8):3168-75

Used symbols:


Temperature limitation

Catalogue number

In Vitro Diagnostic Medical Device

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet