PROVIDER OF CE-CERTIFIED LC-MS/MS DIAGNOSTIC KITS

MASSDETECT™ PETH

INSTRUCTIONS FOR USE
FOR THE IN VITRO DETERMINATION OF PETH 16:0/18:1 IN BLOOD

CE

CE-IVD label according to European Directive 98/79/EC

redhot diagnostics AB
Forskargatan 20J
SE-15136 Södertälje
Sweden

www.redhotdiagnostics.com
contact@redhotdiagnostics.com

Organization no: 556861–7731
Bank account: 77980-1

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1. PHOSPHATIDYLETHANOL, MASSDETECT PETH LC-MS/MS KIT

Art. No. 50-2002, 50-2003 MassDetect™ PEth 200 respectively 500 analyzes including column.

Art. No. 50-2001, 50-2004 MassDetect™ PEth 200 respectively 500 analyzes, replacement kit.

Risk class 1 according to IVDD, and risk class A according to IVDR

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Manufacturer

redhot diagnostics AB
Forskargatan 20J
SE-151 36 Södertälje
Sweden
www.redhotdiagnostics.com

2. INTENDED USE

The described LC-MS/MS application is intended for the quantitative determination of phosphatidylethanol (PEth) 16:0/18:1 in blood of humans.

The method is for monitoring long term alcohol intake to total abstinence.

The method is intended to be performed by a laboratory professional in a clinical laboratory.

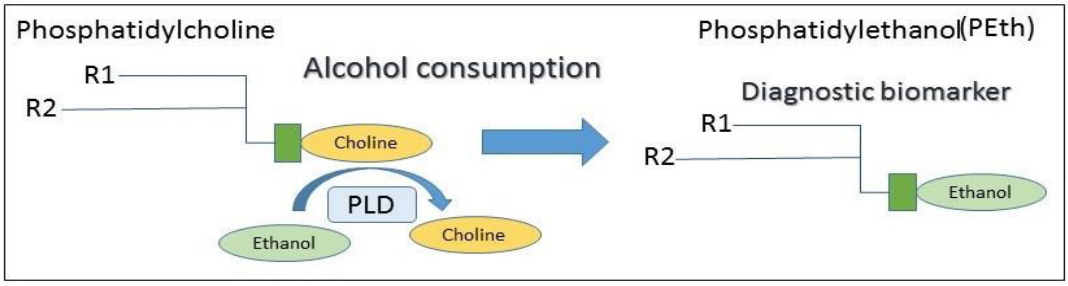
For in vitro diagnostic use only.

3. INTRODUCTION

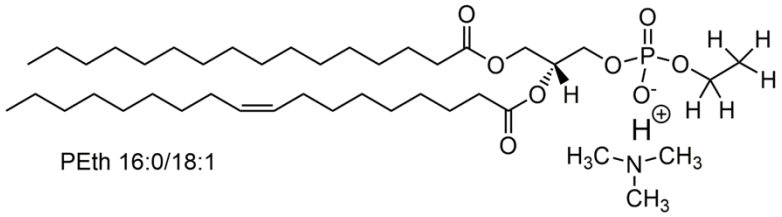
Phosphatidylethanol (PEth) in blood is a biomarker for previous alcohol consumption (1). Alcohol

consumption can give rise to many different symptoms (2). An analyze of PEth concentrations in the blood of a patient could thus be a start for an investigation of a disease.

PEth is an unnatural phospholipid formed only in the presence of ethanol, giving a theoretical diagnostic specificity of 100% as a biomarker for alcohol consumption.



The principle for in-vivo generation of PEth depends on rearrangement of phosphatidylcholine in presence of phospholipase D.



PEth-16:0/18:1 is the most abundant individual form of PEth-homologues (3) but there are inter individual variations (4). PEth-16:0/18:1 has been accepted as the best form for use in quantitative LC-MS/MS analysis to estimate the level of alcohol consumption in patients. It has been shown that there is a direct correlation between alcohol consumption and the levels of PEth in blood (5, 6).

PEth is the only biomarker that gives a direct correlation to alcohol consumption, and it has been shown to be a more sensitive indicator of alcohol consumption than other markers (7, 8). The half-life of PEth in circulation is three to eight days, which means that PEth can be detected up to 3 weeks after ethanol has been cleared from the body.

The PEth levels in blood has shown to be a very useful in where alcohol consumption is difficult to assess (9).

The test developed by redhot diagnostics is a quantitative test which is robust with high specificity and sensitivity. The method is developed as a manually performed test.

4. PRINCIPLES OF THE PROCEDURE

Phosphatidylethanol is extracted from blood by addition of 150 µL extraction buffer, containing an internal standard (deuterated phosphatidylethanol) to 20 µL of sample. After thorough mixing, the tube is centrifuged, and an aliquot of the supernatant is injected in the LC-MS system. The components are separated on a column (art.no. 52015) using a binary gradient. The effluent is monitored with electrospray ionization mass spectrometry using multiple reaction monitoring (MRM) to follow the respective characteristic transitions for PEth and the internal standard. The ratio between the chromatographic peak areas for PEth and the internal standard are used to quantify the concentration of PEth in the samples.

5. WARNING AND PRECAUTIONS

Materials included in this kit should not be used past the expiration date on the kit label.

Reagents or substrates included in this kit should not be mixed or substituted with reagents or substrates from other kits.

Precautions should be taken when the extraction solution and mobile phases are handled.

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6. HEALTH AND SAFETY PRECAUTIONS

Wear personal protective equipment such as safety glasses, gloves, laboratory coats when blood samples are handled.

Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to discard residues in accordance with local laboratory regulations.

7. KIT CONTENTS

Art. no. 50-2001, 200 determinations, including column

Art. no. 50-2002, 200 determinations, replacement kit

Component	Quantity
Calibrators in blood 0, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0 and 2.0 µM	7 x 0.4 mL
D5-PEth 16:0/18:1, 4.5 nmol	0.5 mL
Extraction solution	44.5 mL
Tuning solution, 1 µM	0.5 mL
Column	1 pcs.

Art. no. 50-2003, 500 determinations, including column

Art. no. 50-2004, 500 determinations, replacement kit

Component	Quantity
Calibrators in blood 0, 0.05, 0.1, 0.2, 0.5, 1.0 and 2 .0 µM	7 x 0.4 mL
D5-PEth 16:0/18:1, 4.5 nmol	2 x 0.5 mL
Extraction solution	2 x 44.5 mL
Tuning solution, 1 µM	0.5 mL
Column	1 pcs.

8. KIT STORAGE CONDITIONS

The extraction solution should be stored at +2-8^{o}C.
The Calibrator, internal standards and extraction solution including internals standard should be stored at -20^{o}C

9. MATERIALS REQUIRED BUT NOT SUPPLIED

1. LC-MS/MS-Equipment
2. Mobile phase A
3. Mobile phase B
4. Vortex
5. Pipettes
6. Centrifuge
7. Vials
8. Appendix 1 lists products that are available for the PEth method.

10. PREPARATION OF MOBILE PHASES

Mobile phase A

5 mM ammonium acetate in water	Preparation of 1 000 mL
Ammonium acetate	1 mL 5 M ammonium acetate
Milli-Q water	999 mL

Mobile phase B

10% 2-propanol in 90% methanol	Preparation of 1 000 mL
Methanol	900 mL
2-propanol	100 mL

11. CHROMATOGRAPHIC CONDITIONS LC-MS/MS METHOD

Listed as an example for Sciex API5500

Instrument	Sciex API5500
Ionization	Electrospray
Scan Type	MRM
Polarity	ESI-
Curtain Gas	10
Collision Gas	8
Ion Spray Voltage (kV)	-4500
Temperature	500
Ion Source Gas 1	40
Ion Source Gas 2	40
DP (declustering potential)	150 – 220
CE (collision energy)	38 - 40

12. TRANSITION

Molecule	Transitions
PEth 16:0/18:1	701.5 > 281.2, 255.6
D5-PEth 16:0/18:1	706.5 > 281.2, 255.6

Fragment 281 is used for quantification and 255 as a qualifier.

13. LC-GRADIENT

Flow rate: 0.4 mL/min

Analysis time: 3.5 min

Time [min]	Mobile phase A [%]	Mobile phase B [%]
0-0.2	30	70
0.2-1.5	0	100
1.5-2.5	0	100
2.5-2.6	30	70

14. WASH SOLUTION

Due to the sticky properties of PEth we recommend that the autosampler is washed regularly, for example with 2-propanol, methanol, and ammonium acetate.

15. STARTUP – OPTIMIZATION OF PARAMETERS FOR THE ANALYTES

Use the included PEth tuning solution to find the exact transitions for the MRM traces when setting up the kit for the first time.

PEth ionize and fragment easily and depending on instrument and parameters, the MS response might be too high for the electron multiplicator. If so, decrease the injection volume and/or dilute the samples.

Check the accuracy of the mass scales after annual preventive maintenance of the mass spectrometer, and after all other manipulations which can affect the accuracy of the mass scales.

16. SAMPLES – STORAGE AND TRANSPORTATION CONDITIONS

Analysis is performed in blood: Use only EDTA tubes. NOTE! Do not centrifuge! Mix the sample in a blood cradle or by turning it upside down ten times immediately after sampling to prevent clots in the tube.

If transport to the laboratory cannot be arranged immediately, the sample must be stored in a refrigerator. Shelf life in the fridge: 1 week. Transport takes place without refrigeration.

For longer time storage -20^{0}C is recommended.

Extracted samples are stored at +2-8^{o}C with a shelf-life of two weeks.

Allow samples and reagents to reach room temperature before use.

17. PREPARATION OF EXTRACTION SOLUTION

Centrifuge the ampule containing internal standard D5-PEth 16:0/18:1 (2000 RCF, 2 min), open the ampule and add 0.5 ml extraction solution, transfer the solution in its entirety to the extraction bottle, repeat 2 times to quantitatively transfer the internal standard to the Ext. sol. bottle. The final concentration of D_5- PEth 16:0/18:1 internal standard is 0.1 µM.

18. SAMPLE PREPARATION

1. To 150 µL EXT add 20 µL blood, vortex thoroughly for 2 x 5 sec.
2. Centrifuge the tubes at approximately 16400 RCF for 10 min. If possible, use at 10°C to get a better precipitation/pellet.
3. Transfer 120 µL of the supernatant to an autosampler vial, place the vial in the autosampler, inject 5 µL of the sample into the instrument.

19. CALIBRATOR CURVE

The calibrator curve is ready-to-use. See sample preparation pkt. 18

20. DETERMINATION OF PETH CONCENTRATIONS

For each calibrator concentration, the peak area of the analyte is divided by the corresponding peak area of the internal standard. These ratios are plotted against the calibrator concentrations to calculate the calibrator curve equation, which is used to determine the PEth concentration of the sample. First order linear regression weighted by 1/x is preferred.

Figure 2. Example of calibration curve based on chromatograms from 701.5 -> 281.2

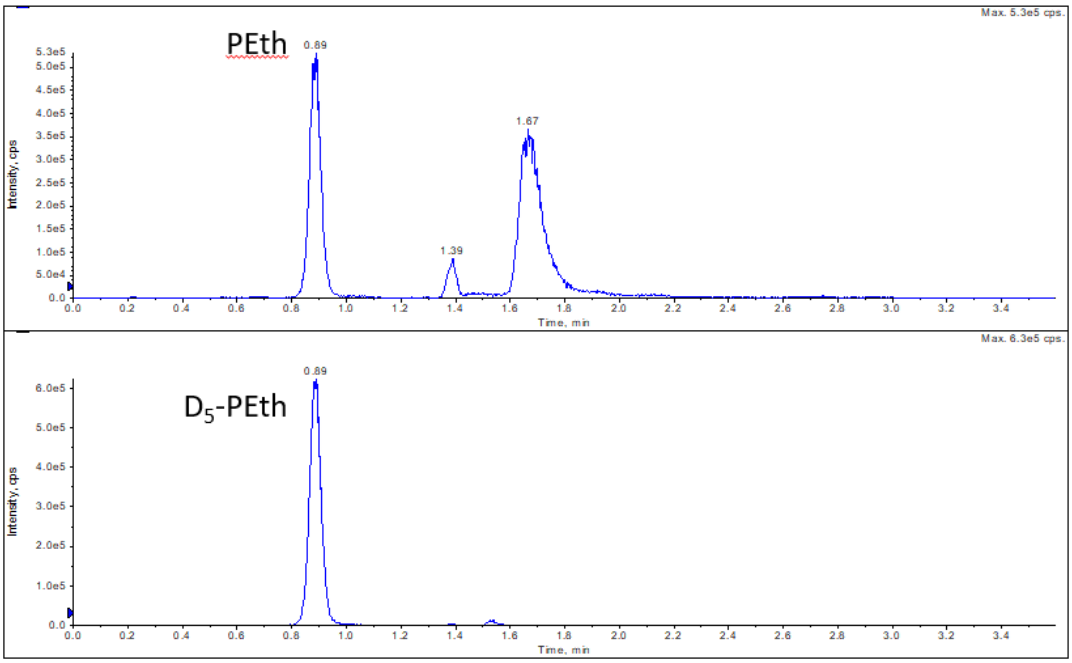
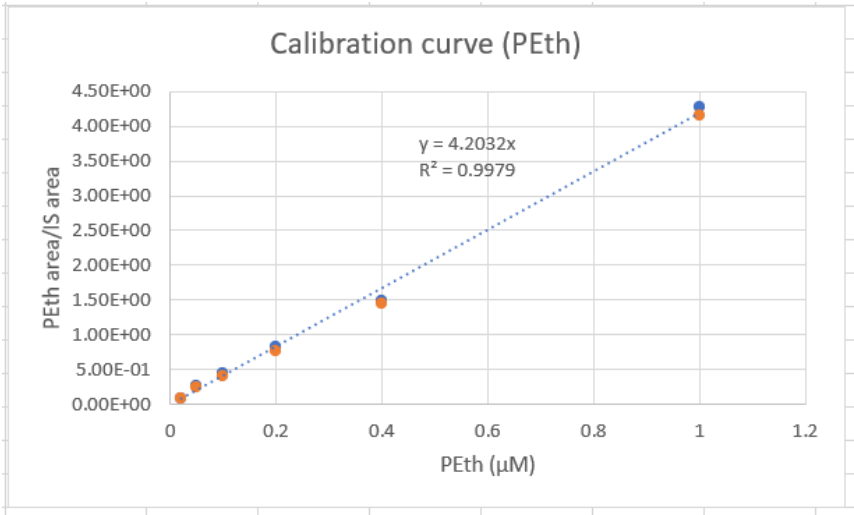


Figure 3. Example of control sample at 0.5 µM

21. QUALITY CONTROL PROCEDURES

Control samples should be analyzed together with each batch of samples. Results generated from the analysis of control samples should be evaluated by statistical methods to ensure that the method shows accurate results. It is recommended to monitor the peak areas of the internal standard for all batches.



The peak area should be consistent, and any inconsistency or systematic decrease of the internal standard can indicate interference, carryover effects and/or hardware related issues, such as contaminated column or ion source. Individual outliers can indicate issues with the sample or the preparation of the sample.

22. PERFORMANCE CHARACTERISTICS

Performance characteristics may vary depending on the instrument used.

Measuring range

0.02 – 1.0 µM

Lowest detection level

0.02 µM

Reproducibility

PEth [µmol/L]	Intra Assay CV (%) (n=10)
0.034	16
0.27	10
0.74	5

23. REFERENCE INTERVAL FOR PETH

According to Swedish Transport Agency (10)

PEth [µmol/L]	PEth [µg/L]	Comments
< 0.03	<20	None or low
0.03 – 0.3	20 – 200	Moderate Intake
> 0.3	>200	Large intake

24. REFERENCES

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