Phosphatidylethanol (PEth) ELISA

K-5500Q (96 tests)

Support: echelon@echelon-inc.com

Description: ELISA to detect the alcohol biomarker, PEth, extracted from dried blood spot (DBS) cards

Storage: Upon receipt, store PEth ELISA and kit components at 4°C.

Materials Provided

Catalog #	Description	Quantity	Volume
K-5001	Detection Plate	1 plate	
K-5503	Sample Diluent	1 bottle	25 mL
K-5504Q	Antibody Diluent	1 bottle	10 mL
K-5505	Anti-PEth Ab	1 vial	70 µL
K-PBST2	10x PBS-T Buffer	1 bottle	20 mL
K-TMB1	TMB Solution	1 bottle	12 mL
K-STOPt	1 N H_2SO_4 Stop Solution	1 bottle	8 mL
------	Pre-incubation Plate (Yellow)	1 plate	
------	Acetate Plate Sealer	2 seals	
K-5510	PEth Control Card	1 card	

Quick Protocol

Extract PEth from PEth Control Card and DBS card samples.

Reconstitute the extracted PEth Controls and samples for 1 hour.

Combine PEth Controls or samples with Anti-PEth ab in incubation plate. Incubate at room temperature for 1 hour with shaking.

Transfer samples from incubation plate to Detection Plate. Incubate at room temperature for 1 hour with shaking.

Discard & wash 3 times with Wash Buffer.

Add TMB Solution. Incubate for 30 minutes at room temperature, protecting from light.

Add 1 N H_2SO_4 Stop Solution. Read absorbance at 450 nm.

Analyze Data

Additional Materials Provided by User

- Speedvac (optional)
- Plate reader for reading absorbance at 450 nm
- Plate shaker
- Methanol (for sample extraction)
- 3.2 mm or 6 mm (standard size) round hole punch

We strongly recommend using the entire assay in a single application, as opened and reconstituted reagents are less stable. Refer to assay notes for additional storage information.

This product is intended for use with samples extracted from dried blood spot (DBS) cards. We recommend using only the procedure outlined in this protocol.

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Background

Phosphatidylethanol (PEth or Ptd-OH) is a direct biomarker of alcohol ingestion formed in the blood only when ethanol has been consumed. It is specific and can report previous alcohol consumption beginning a few hours after a drinking event and continuing 2-3 weeks later. Sensitive detection by mass spectrometry using whole blood or dried blood spot (DBS) cards is becoming generally accepted as the gold-standard for detecting past alcohol consumption. Echelon's PEth ELISA is the first commercially available immunoassay for detection of PEth in blood.

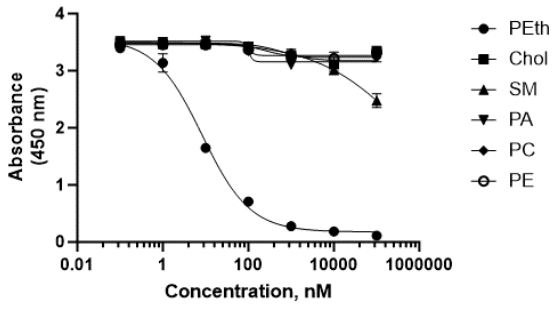
Assay Design

The assay is a competitive immunoassay where PEth present in a sample extract competes with PEth on a plate for binding to a specific HRP-conjugated antibody. Thus, the assay signal is inversely proportional to the amount of PEth present in the sample. This assay is designed for dried blood spot card samples. The antibody recognizes all major PEth molecular species. The assay provides researchers a quick and robust method to screen for PEth in whole blood spotted on DBS cards.

Assay Notes

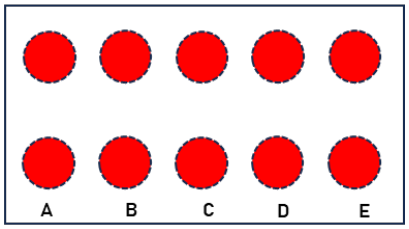
1. Use Milli-Q or equivalent laboratory grade water free of contaminants.
2. Bring all reagents to room temperature before use, except the Anti-PEth Ab (K-5505) which should remain at 4 °C or on ice until use.
3. If a plate shaker is not available, tap plate to mix after all reagents are added. The plate can be incubated without shaking but the user should expect a higher coefficient of variation.
4. Incubation times are optimized. Deviation from these times may result in aberrant signals.
5. This assay is optimized for measuring PEth from whole blood spotted on DBS cards. Serum and plasma specimens are NOT compatible with this assay. If the specimen is collected in a tube via venipuncture, we recommend pipetting 50 µL of whole blood on a DBS card, letting the sample dry 3+ hours before punching and extracting as described in the protocol. Storage of whole blood prior to spotting DBS cards can affect the results. For best results, avoid freezing or storage of any whole blood samples over 1-2 weeks at 4°C.
6. The antibody in this assay does not cross react with other major lipid species found in blood (Figure 1).

Figure 1. Assay specificity with other major lipids found in red blood cell erythrocytes. PEth = phosphatidylethanol, Chol = cholesterol, SM = sphingomyelin, PA = phosphatidic acid, PC = phosphatidylcholine, PE = phosphatidylethanolamine.



7. The PEth Control Card (K-5510) consists of whole blood with five PEth levels spotted on DBS Cards and is utilized for the standard curve. These standards are to be run in parallel to the user's samples and should experience the same extraction treatment conditions. These are the preferred reference controls for the assay. Two spots are supplied for each PEth Control allowing the option to run additional PEth Control replicates.

K-5510 "PEth Control Card"



8. We strongly recommend using the entire assay in a single application. However, a Detection Plate (K-5001) with removable strip-wells and extra assay reagents are provided, allowing the flexibility to run the assay twice.
9. When running the assay in two separate applications, please:
   a. Modify the Anti-PEth Ab Dilution accordingly (step 4 of ELISA protocol).

   For example: To run a half plate add 30 µL Anti-PEth Antibody to 4.5 mL Antibody Diluent (K-5504).

   b. Return remaining assay reagents to suggested storage temperatures after the first use.

PEth Extraction / Sample Preparation Protocol

Process DBS Samples prior to running ELISA. Please read the entire PEth Extraction & ELISA Protocols before beginning the experiment.

1. Turn the DBS cards over and visually select a punch target completely saturated with blood. Using the hole puncher, punch four 3.2 mm or one 6 mm circles, each approximating 12 µL of whole blood, for each control and sample.
2. Place the punched circles into properly labeled polypropylene microcentrifuge tubes and add 500 µL methanol. Small lab forceps or tweezers may be helpful.
3. Cap the tubes, vortex 5 minutes, and incubate for 50 minutes at room temperature. Vortex the tubes again for 5 minutes at the end of the incubation.
4. Transfer 400 µL of the methanol extract to a fresh polypropylene tube without disturbing the punches.
5. Completely evaporate the methanol from the extracted samples and controls. This can be accomplished in a speed-vacuum for 60-90 minutes **WITHOUT HEATING** or allowing sample tubes to stand open overnight in a fume hood, biological safety cabinet, or protected clean environment. Other similar drying methods that avoid heating may be used but have not been evaluated in this assay. Ensure the samples are dry. Analyze directly by ELISA or store dried samples at -20°C until use.

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ELISA Protocol

This protocol is written for running the entire plate. Please refer to Assay Notes #8-#9 if running a partial plate.

1. Bring all reagents to room temperature before use, except the Anti-PEth Ab (K-5505). Leave this at 4 °C or keep on ice until used (step 4).
2. Reconstitute the dried extracted PEth controls and samples with 150 µL Sample Diluent (K-5503). Vortex for 1 minute, let sit at room temperature for 1 hour, then vortex again for 1 minute to fully reconstitute the PEth controls and samples.
3. Prepare Wash Buffer by adding 20 mL 10x PBS-T Buffer (K-PBST2) to 180 mL H_2O. Mix well. Store at room temperature.
4. Dilute Anti-PEth Ab (K-5505) by adding 60 µL Anti-PEth Ab (K-5505) to 9.0 mL Antibody Diluent (K-5504). Mix gently but thoroughly. Keep at room temperature until use. See assay note #9 if the entire assay is not run in a single application.
5. Combine the PEth controls and reconstituted samples (step 2) with the diluted Anti-PEth Ab (step 4) in the yellow pre-incubation plate (see suggested layout below) as follows:
   a. Vortex the reconstituted PEth Controls and samples.
   b. For Blank (Well F1), add 80 µL Sample Diluent (K-5503), and 80 µL Antibody Diluent (K-5504).
   c. Add 80 µL of reconstituted PEth controls to Wells A1 to E1.
   d. Add 80 µL of reconstituted samples to the remaining wells.
   e. Add 80 µL of the diluted Anti-PEth Ab (step 4) into all wells except Blank (Well F1).
6. Seal the plate with an acetate plate sealer and incubate with gentle shaking for 1 hour at room temperature. See Assay Note #3 if plate shaker is not available.
7. After incubation, use a multichannel pipette to pre-mix solutions three times before transferring 100 µL to the Detection Plate (K-5001). Cover plate with a new acetate plate seal and incubate on plate shaker for 1 hour at room temperature.
8. After incubation, discard solutions and wash wells three times with 200 µL/well of Wash Buffer (Step 3).
9. Add 100 µL/well TMB Solution (K-TMB1). Cover plate with acetate plate sealer and incubate for 30 min in the dark. Do not shake.
10. Carefully remove the acetate plate sealer. Add 50 µL/well 1N H_2SO_4 Stop Solution (K-STOPt) to stop reaction.
11. Read the plate at 450 nm.

Suggested Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Control A	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35	Sample 43	Sample 51	Sample 59	Sample 67	Sample 75	Sample 83
B	Control B	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36	Sample 44	Sample 52	Sample 60	Sample 68	Sample 76	Sample 84
C	Control C	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37	Sample 45	Sample 53	Sample 61	Sample 69	Sample 77	Sample 85
D	Control D	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38	Sample 46	Sample 54	Sample 62	Sample 70	Sample 78	Sample 86
E	Control E	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39	Sample 47	Sample 55	Sample 63	Sample 71	Sample 79	Sample 87
F	Blank	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40	Sample 48	Sample 56	Sample 64	Sample 72	Sample 80	Sample 88
G	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33	Sample 41	Sample 49	Sample 57	Sample 65	Sample 73	Sample 81	Sample 89
H	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34	Sample 42	Sample 50	Sample 58	Sample 66	Sample 74	Sample 82	Sample 90

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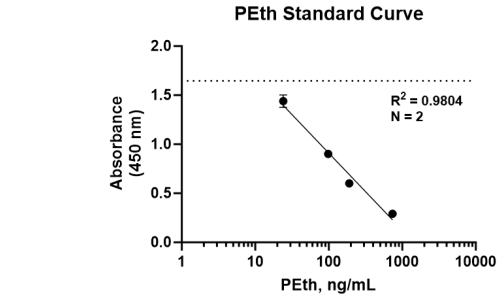
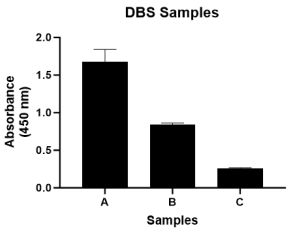
Data Analysis

1. Refer to Certificate of Analysis (COA) for lot specific PEth Control concentrations.
2. Plot the PEth Controls concentration in ng/mL on x-axis as log scale and the measured ELISA optical density (O.D.) at 450 nm as y-axis.
3. Fit data with linear regression to generate a PEth Standard Curve.
4. Interpolate samples against the PEth standard curve. An example of the PEth standard curve and sample interpolation is shown in Figure 3.

Notes: We recommend using semi-log linear regression for data analysis. If other curve fit is desired, ensure curve fit analysis is consistent between experiments for best results.

Figure 3. Example of PEth Standard Curve and Sample Competition in PEth ELISA (K-5500Q)





DBS Sample	LC/MS Values	PEth ELISA Values (n = 2)
A	≤ 8 ng/mL	11 ± 5 ng/mL
B	79 ng/mL	122 ± 7 ng/mL
C	737 ng/mL	681 ± 28 ng/mL

References

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Related Products

Catalog #	Product
PEth Binding Reagents	
Z-PEth	Anti-Phosphatidylethanol Antibody
P-BPEth	Phosphatidylethanol (PEth) Beads
Lipids	
L-6017	17:0, 18:1 Phosphatidylethanol
L-6018	18:1,18:1 Phosphatidylethanol
L-6019	16:0,18:1 Phosphatidylethanol
L-6020	16:0, 18:2Phosphatidylethanol
L-60F18	BODIPY-FL-Phosphatidylethanol
L-60N16	NBD-Phosphatidylethanol
L-60B16	C12-Biotin, 16:0-Phosphatidylethanol
L-60B18	C12-Biotin, 18:0-Phosphatidylethanol
L-6051	d5-POPEth (deuterated)
L-6052	d5-PLPEth (deuterated)
L-6053	d5-SOPEth (deuterated)
L-6054	d5-SLPEth (deuterated)
L-6055	d5-PAPEth (deuterated)

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