Technical Bulletin

Phospholipid Quantification Assay Kit (Colorimetric/Fluorometric)

Sufficient for 200 colorimetric or fluorometric tests

CS0001

Product Description

Phospholipids are a class of lipids, which constitute a major component of cell membranes and play important roles in signal transduction. Most phospholipids have a diglyceride structure with a phosphate group and one choline moiety. Phosphatidylcholine-containing phospholipids have been estimated to be present in serum at 0.2-2.5 mM (50-200 mg/dL).[1]

For analysis of phospholipids, enzymatic methods have been developed that use different enzymes in sequence:[2-4]

- Release of choline from phospholipids by phospholipase D
- Oxidation of the released choline by choline oxidase

The hydrogen peroxide released from the oxidation of choline then reacts with peroxidase and appropriate substrates to give end products that can be detected by either fluorometric or colorimetric methods.

The Phospholipid Quantification Assay Kit provides a simple, quick, and high-throughput assay to measure choline-containing phospholipids in biological samples. In this assay, phospholipids are enzymatically hydrolyzed to release choline, which is determined using choline oxidase and an H_2O_2-specific dye.

This kit allows for either fluorometric or colorimetric detection, to allow maximum flexibility and a large detection range. The linear ranges are as follows:

- Fluorescence assay: 0.1-0.5 nmol
- Colorimetric assay: 1-5 nmol

Several publications[5-7] and dissertations[8] have cited use of this kit in their research protocols.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Components

This kit contains sufficient reagents for 200 assays in 96-well plates.

Component	Component Number	Amount	Cap Color/ Container Information
Assay Buffer	CS0001A	30 mL	White cap/ bottle
Probe	CS0001B	220 µL	Brown vial
Hydrolysis Enzyme	CS0001C	1 vial	Red cap/vial
Development Mix	CS0001D	1 vial	Green cap/vial
Standard	CS0001E	220 µL	Yellow cap/vial

Component Information

- Assay Buffer (CS0001A): Ready-to-use. This component may be stored either at –20 °C or at 2-8 °C. Equilibrate to room temperature before use.
- Probe (CS0001B): Ready-to-use. Store at –20 °C, protected from light. Equilibrate to room temperature before use. During the experiments, do **not** keep on ice.

  Note: To reduce the Probe background in the fluorescence assay, dilute an aliquot of the Probe 10-fold with Assay Buffer, immediately prior to use.

- Hydrolysis Enzyme (CS0001C): Store at –20 °C. Reconstitute with 440 µL of ultrapure water. Mix well by pipetting. Keep on ice while in use. Unused material should be aliquoted and stored at –20 °C.
- Development Mix (CS0001D): Store at –20 °C. Reconstitute with 440 µL of ultrapure water. Mix well by pipetting. Keep on ice while in use. Unused material should be aliquoted and stored at –20 °C.
- Standard (CS0001E): Ready-to-use 50 mM Standard solution (molecular mass 768 Da). Store at –20 °C. Keep on ice while in use. Unused material should be aliquoted and stored at –20 °C.

Equipment Required, But Not Provided

- 96-well flat-bottom plates:
  - Use black plates for fluorescence assays.
  - Use clear plates for colorimetric assays.
- Fluorescence (λ_{ex} = 535 nm / λ_{em} = 587 nm) or spectrophotometric (570 nm) multiwell plate reader.

Storage/Stability

The kit is shipped on dry ice. Upon receipt, store all components at –20 °C, protected from light. Upon thawing, the Assay Buffer can be stored at 2-8 °C. The unopened kit is stable for 2 years as supplied.

Preparation Instructions

- Use ultrapure water (≥18 MΩ × cm resistivity at 25 °C) for the preparation of reagents and standards.
- Thaw all components before use.
- Briefly centrifuge vials before opening.

Procedure

- All samples and standards should be run in duplicate.
- A fresh set of standards should be prepared for every use.
- **For convenience, an Excel-based calculation sheet is available on the Product Detail Page. Use this sheet to calculate the amounts of reagents required, as well as to calculate the test results.**

Standards for Colorimetric Detection

1. Dilute the Standard 100-fold to a final concentration of 0.5 mM: 10 µL of the Standard solution with 990 µL of ultrapure water.
2. Add 0, 2, 4, 6, 8, and 10 µL of the 100-fold diluted Standard solution into a 96-well plate, generating 0 (blank), 1, 2, 3, 4, and 5 nmol/well standards.
3. Complete the volume to 50 µL with ultrapure water (see Table 1).

Table 1. Standards for the Colorimetric assay

100-fold diluted Standard volume*	Ultrapure Water volume*	Final phospholipids amount per well (nmol)*
0 µL (Standard Blank)	50 µL	0
2 µL	48 µL	1
4 µL	46 µL	2
6 µL	44 µL	3
8 µL	42 µL	4
10 µL	40 µL	5

* Work in duplicate

Standards for Fluorometric Detection

1. Dilute the Standard 1,000-fold to a final concentration of 0.05 mM. We recommend preparation of the 0.05 mM Standard by serial dilution:
   1.1. Prepare an initial 100-fold dilution, using 10 µL of the Standard solution with 990 µL of ultrapure water.
   1.2. Dilute 50 µL of the 100-fold diluted Standard solution with an additional 450 µL of ultrapure water, to obtain a 1,000-fold dilution at 0.05 mM.
2. Add 0, 2, 4, 6, 8, 10 µL of the 1,000-fold diluted Standard solution into a 96-well plate, generating 0 (Blank), 0.1, 0.2, 0.3, 0.4, and 0.5 nmol/well standards.
3. Complete the volume to 50 µL with ultrapure water (see Table 2):

Table 2. Standards for the Fluorescence assay

1,000-fold diluted Standard volume*	Ultrapure Water volume*	Final phospholipids amount per well (nmol)*
0 µL (Standard Blank)	50 µL	0.0
2 µL	48 µL	0.1
4 µL	46 µL	0.2
6 µL	44 µL	0.3
8 µL	42 µL	0.4
10 µL	40 µL	0.5

* Work in duplicate

Sample preparation

- Thiol (SH) group-containing reagents (such as DTT or mercaptoethanol), sodium azide, EDTA, and sodium dodecyl sulfate (SDS) may interfere with this assay. These reagents should be avoided in sample preparation.
- Add samples (such as serum, plasma) directly to the sample wells in the 96-well plate. Bring the samples to a final volume of 50 µL with Assay Buffer.
- For unknown samples, it is suggested to test several sample dilutions using Assay Buffer, to ensure that the readings are within the linear range of the Standard curve.
- Choline present in the sample can generate background. To control for choline background, include a choline control sample for each sample by omitting the Hydrolysis Enzyme in the Reaction Mix.

Reaction Mixes

- Set up the Reaction Mixes according to Table 3.
- 50 µL of the appropriate Reaction Mix is required for each reaction (well).
- Multiply the volumes in Table 3 according to the number of wells in the assay.

Table 3: Reaction mixes, one per well

Reagents	Samples and standards per well	Choline control** per well
Assay Buffer	45 µL	47 µL
Probe***	1 µL	1 µL
Hydrolysis enzyme	2 µL	0 µL
Development mix	2 µL	2 µL

** If required

*** For the **fluorescence assay**, dilute the Probe 10-fold with Assay Buffer prior to addition to the Reaction Mix.

Assay reaction

1. Add 50 µL of the appropriate Reaction Mix to each of the standard and sample wells.
2. Mix well using a horizontal shaker or by pipetting.
3. Incubate the reaction for 30 minutes at 37 °C. Measurement up to 60 minutes is possible. Protect the plate from light during the incubation.
4. For colorimetric assays, measure the absorbance at 570 nm.
5. For fluorometric assays, measure fluorescence intensity at λ_{ex} = 535 nm / λ_{em} = 587 nm.

Results

Calculations

1. Subtract the Blank value (no Standard) from all Standard and Samples values.
2. Plot the absorbance or fluorescence measured for each Standard against the Standard concentrations.

3. Determine the linear regression equation, and use it to calculate the phospholipid concentration of the sample:

[(Sample)/(Sample volume)] × DF = nmol/µL (mM)

Where:

Sample = Amount of phospholipids in unknown sample (nmol), calculated from the standard curve

Sample volume = Sample volume added into the wells (50 µL)

DF = Sample dilution factor (if sample is not diluted, the DF value is 1)

The molecular mass of the phospholipid is 768 Da. Therefore, to convert the concentration from mM to ng/µL, multiply the result (in mM) by 768 g/mol (768 ng/nmol).

Sample calculation

- Sample = 5.84 nmol (from standard curve)
- Sample volume = 50 µL
- DF = 20
- Concentration of phospholipids in the sample:

[(5.84 nmol)/(50 µL)] × 20 = 2.34 nmol/µL

2.34 nmol/µL × 768 ng/nmol = 1,797 ng/µL

If the sample choline control (essentially free choline, without Hydrolysis Enzyme) is significant, subtract the choline control from the sample reading.

References

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