Version 2 Last updated 28 June 2022

ab239717 Glycerophosphorylcholine Assay Kit

For the measurement of GPC content/concentration in various biological samples.

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1. Overview

The Glycerophosphorylcholine Assay Kit (ab239717) allows a quick and sensitive means to determine GPC concentration in a biological sample. The assay is robust and specific, with a GPC enzyme mix converting GPC in the sample into an intermediate that is then utilized to quantitatively generate signal using the GPC Developer mix and GPC Probe.

The assay is rapid, simple, high throughput compatible, and can detect GPC concentrations as low as 0.1 µM in tissue lysates and other samples such as breast milk and seminal plasma.

2. Protocol Summary

Prepare all samples, controls and standards as instructed.

Prepare the standard curve using the 10 mM GPC standard. For a colorimetric assay dilute to a 1mM working solution. For a Fluorometric assay further dilute to a 50 µM GPC Standard solution.

Create the reaction mix and add 50 µL to each well containing standards and test samples.

Incubate the plate for 30 minutes at 37°C, in the dark.

Measure the absorbance (570 nm) or fluorescence (Ex/Em = 535/587 nm) of all reaction, sample background and standard curve wells in endpoint mode.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
GPC Assay Buffer	25 mL	-20°C
GPC Enzyme Mix	1 vial	-20°C
GPC Developer	1 vial	-20°C
GPC Probe (in DMSO)	200 µl	-20°C
Sample clean-Up Mix	1 vial	-20°C
GPC standard (1 µmole)	1 vial	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Clear or Black (Fluorometric only) 96-well plate with flat bottom.
- Multi-well spectrophotometer.
- 10 kDa Spin column.
- Deproteinizing Sample Preparation Kit.

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 GPC Assay Buffer:

Allow to warm to room temperature prior to use. Store at 4°C, protected from light.

6.2 GPC Probe:

Provided as a solution in DMSO. Store at -20°C, protected from light. Prior to use, warm solution to room temperature. After use, promptly retighten cap to minimize adsorption of airborne moisture.

6.3 GPC Enzyme Mix:

Reconstitute each vial with 220 µl GPC Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use and use reconstituted aliquots within two months.

6.4 GPC Developer:

Reconstitute each vial with 220 µl GPC Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use and use reconstituted aliquots within two months.

6.5 Sample clean-Up Mix:

Reconstitute each vial with 220 µl GPC Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use and use reconstituted aliquots within two months.

6.6 GPC Standard (1µmole):

Reconstitute with 100 µl ddH2O and mix thoroughly to generate a 10 mM GPC Standard solution. Aliquot and store at -20°C. Use within two months.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 **For Colorimetric Assay:** Dilute the 10 mM GPC Standard by combining 10 µl with 90 µl dH2O to generate a 1 mM solution.

7.2 **For the fluorometric assay:** Further dilute the 1 mM solution by adding 50 µl to 950 µl dH2O, yielding a 50 µM GPC Standard working solution.

7.3 Add 0, 2, 4, 6, 8 and 10 µl of the 1 mM working solution into a series of wells in a black 96-well plate to generate 0, 2, 4, 6, 8 and 10 nmol GPC standard for **colorimetric detection**.

7.4 For **fluorometric detection** a Add 0, 2, 4, 6, 8 and 10 µl of the 50 µM working solution to generate 100, 200, 300, 400 and 500 pmole per well standard.

7.5 Bring the total volume of each well to 50 µl with GPC Assay Buffer.

Standard #	1 mM GPC Working solution (µL)	GPC Assay Buffer (µL)	GPC standard (nmol)
1	10	40	10
2	8	42	8
3	6	44	6
4	4	46	4
5	2	48	2
6	0	50	0

Table 1: Standard curve preparation for a colorimetric assay.

Standard #	50 µM GPC Working solution (µL)	GPC Assay Buffer (µL)	GPC standard (pmol)
1	10	40	500
2	8	42	400
3	6	44	300
4	4	46	200
5	2	48	100
6	0	50	0

Table 2: Standard curve preparation for a fluorometric assay.

8. Sample Preparation

8.1 For tissues and cultured cells:

- Add 50 µl of ice-cold GPC Assay Buffer per 10 mg of sample (wet weight) or ~1 x 10^6 pelleted cells.
- Homogenize samples on ice using a Dounce homogenizer.
- Centrifuge at 10,000 x g for 5 min at 4°C. Collect the supernatant. Add 2 µl Sample Clean-Up Mix per 100 µl lysate and incubate for 30 min at RT.
- Transfer sample to a 10 kD MWCO filter and filter by centrifugation at 10,000 x g for 10 min. at 4°C. Collect the resultant filtrate and add 2-20 µl to desired wells of a black 96-well plate. Adjust the volume to 50µl per well with GPC Assay Buffer.
- For each sample, prepare identical background control reactions in separate wells.

8.2 Biological fluids:

- Incubate the sample at 37°C for 30 minutes. Clarify with centrifugation at room temperature for 10 min. at 10000 x g.
- Deproteinize the sample using PCA treatment and add 2 µl Sample Clean-Up Mix per 100 µl sample.
- Incubate at 37°C for 30 minutes and then boil the sample for 5 min. Spin down briefly and collect supernatant.
- Prepare duplicate wells, one sample and one background, by adding a volume (2-20 µl) of the supernatant to a black 96-well plate and adjust the volume to 50 µl with GPC Assay Buffer.

ΔNote: Once treated with Sample Cleanup Mix, boiled, and spun down, samples can be stored at -80°C for future experiments.

ΔNote: For unknown samples, we recommend doing a pilot experiment testing several doses to ensure that readings are within the range of the standard curve.

9. Assay Procedure

9.1 Mix enough reagents for the number of assays to be performed, including GPC standard curve wells. For each test sample well, prepare 50 µL Reaction Mix Shown in the table below:

9.2

	Reaction Mix	Sample Background Mix
GPC Assay Buffer	44 µL	46 µL
GPC Enzyme Mix	2 µL	-
GPC Developer Mix	2 µL	2 µL
GPC Probe*	2 µL	2 µL

Δ **Note: *** For fluorometric standards/application, reduce the amount of probe in the reaction mix to 0.4 µl, and adjust the buffer volume to 45.6 µl.

9.3 Mix and add 50 µl of the Reaction Mix to each well containing standards and test samples.

9.4 For Sample Background wells, mix and add 50 µl of the Sample Background Mix to each well.

9.5 **Measurement:** Incubate the plate for 30 min at 37°C, protected from light and read the absorbance (570 nm) or fluorescence (Ex/Em = 535/587 nm) of all reaction, sample background and standard curve wells in endpoint mode.

10. Calculation

10.1 Subtract the 0 GPC Standard reading from all standard curve readings. Plot the background-subtracted GPC Standard Curve and calculate the slope.

10.2 If sample background control reading is significant, subtract the background control reading from its paired sample reading.

10.3 Calculate the corrected absorbance/fluorescence of the test samples $\Delta OD/RFU = OD/RFU_{sample} - OD/RFU_{background}$. Apply the corrected ΔOD (or ΔRFU) value to the GPC Standard Curve to get B pmol GPCs in the well.

Sample GPC Concentration = (B / V) x D = nmol/µl ≡ mM

Where: B = GPC amount from Standard Curve (in pmol)
V = Sample volume added into the reaction well (in µl
D = Sample dilution factor (if applicable)

12. Typical Data

Typical data provided **for demonstration purposes only**.

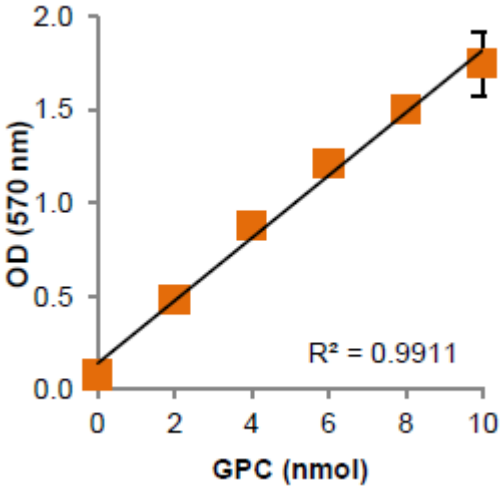

Figure 1. Colorimetric GPC Standard Curve.

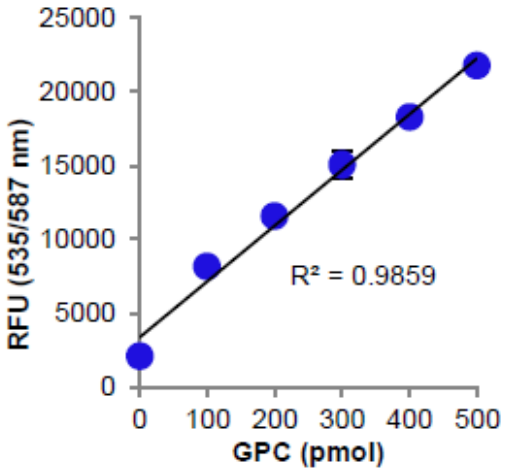

Figure 2. Fluorometric GPC Standard Curve.

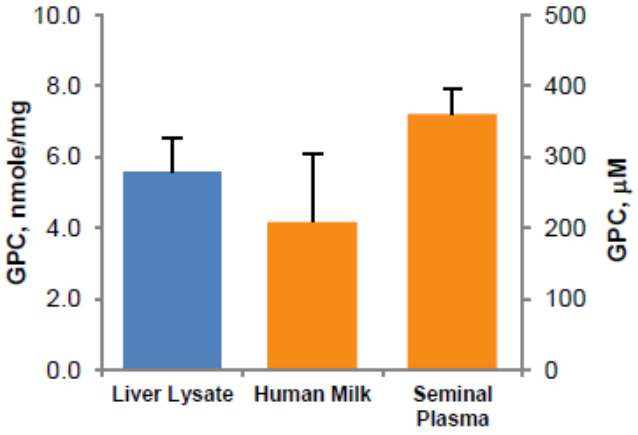

Figure 3: Determination of total GPC concentration in Liver lysate, Human Milk, and Seminal Fluid. For this experiment, 100 mg rat liver was homogenized and prepared according to the kit protocol. Breast Milk and Seminal Plasma were prepped according to the above protocol and treated with Sample Clean-Up Mix. Values were determined with fluorometric measurements and are mean ± standard deviation of at least three independent determinations.

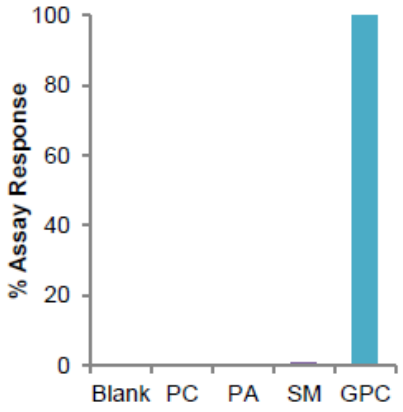

Figure 4: Specificity of the assay; PC = phosphatidylcholine, PA = phosphatidic acid, SM = sphingomyelin, GPC = glycerophosphorylcholine.

13. Notes

Technical Support

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