Glycerophosphorylcholine Assay Kit (Colorimetric/Fluorometric)

(Catalog # BN00678; 100 assays; Store at -20°C)

I. Introduction:

Glycerophosphorylcholine (GPC), also referred to as alpha-GPC or choline alfoscerate, is a small metabolite and potential dementia treatment. GPC is a breakdown product of phosphatidylcholine, and dysregulation of phospholipid metabolism can alter lipid and metabolite concentrations, leading to health problems such as fatty liver, Gaucher's disease, and Tay-Sachs disease. GPC is also component of seminal fluid and breast milk, and a precursor of the neurotransmitter, acetylcholine. As GPC can efficiently cross the blood-brain barrier, it has been hypothesized as a treatment for Alzheimer's disease and other forms of dementia. In such cases, GPC serves as a biomarker for lipid membrane decomposition. Assay Genie's Glycerophosphorylcholine Assay Kit 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 included Sample Clean-Up Mix allows the user to avoid high background from choline, which is present at high levels in many biological fluids. 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.

II. Applications:

- Measurement of GPC content of various tissues/cell extracts
- Determination of GPC concentration in biological fluids

III. Sample Type:

- Tissues (*e.g.* liver, lung)
- Biological fluids (*e.g.* seminal plasma, breast milk)

IV. Kit Contents:

Components	BN00678	Cap Code	Part Number
GPC Assay Buffer	25 ml	WM	BN00678-1
GPC Enzyme Mix	1 vial	Purple	BN00678-2
GPC Developer	1 vial	Green	BN00678-3
GPC Probe (in DMSO)	200 µl	Red	BN00678-4
Sample Clean-Up Mix	1 vial	Blue	BN00678-5
GPC Standard (1 µmole)	1 vial	Yellow	BN00678-6

V. User Supplied Reagents and Equipment:

- Clear or Black (fluorometric only) 96-well plate with flat bottom
- Multi-well spectrophotometer
- 10 kDa Spin Column
- Deproteinizing Sample Preparation Kit (#BN01024 and/or #BN01040)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **GPC Assay Buffer:** Allow to warm to room temperature prior to use. Store at 4°C, protected from light.
- **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.
- **GPC Enzyme Mix GPC Developer, and 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.
- **GPC Standard:** Reconstitute with 100 µl ddH_2O and mix thoroughly to generate a 10 mM GPC Standard solution. Aliquot and store at -20°C. Use within two months.

VII. GPC Assay Protocol:

1. **Sample Preparation:** 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 50 µl per well with GPC Assay Buffer. For each sample, prepare identical background control reactions in separate wells. For biological fluids (e.g. seminal plasma, breast milk), follow the procedure below:
   - **a.** Incubate the sample at 37°C for 30 minutes. Clarify with centrifugation at room temperature for 10 min. at 10000 x g.
   - **b.** Deproteinize the sample using PCA treatment and add 2 µl Sample Clean-Up Mix per 100 µl sample
   - **c.** Incubate at 37°C for 30 minutes and then boil the sample for 5 min. Spin down briefly and collect supernatant.
   - **d.** 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.

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

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

2. **Standard Curve Preparation: For colorimetric assay**: dilute the 10 mM GPC Standard by combining 10 µl with 90 µl dH_2O to generate a 1 mM solution. **For the fluorometric assay**: further dilute the 1 mM solution by adding 50 µl to 950 µl dH_2O, yielding a 50 µM GPC Standard working solution. 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 (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). Bring the total volume of each well to 50 µl with GPC Assay Buffer.
3. **Reaction Mix:** 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 containing:

	Reaction/Standard 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

***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.

Mix and add 50 µl of the Reaction Mix to each well containing standards and test samples. For Sample Background wells, mix and add 50 µl of the Sample Background Mix to each well.

4. **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.
5. **Calculation:** Subtract the 0 GPC Standard reading from all standard curve readings, plot the background-subtracted GPC Standard Curve and calculate the slope. If sample background control reading is significant, subtract the background control reading from its paired sample reading. 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)

GPC MW = 257.2 g/mole

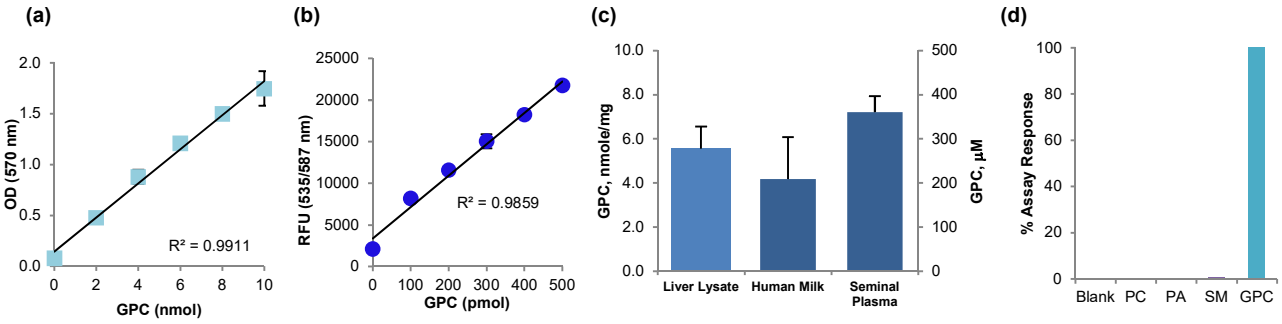

Figure: (a) GPC standard curve, colorimetric and **(b)** fluorometric. **(c)** Determination of total GPC concentration in Liver lysate, Human Milk, and Seminal Fluid. For this experiment, 100 mg rat liver was homogenized and prepared according to the kit protocol. Breast Milk and Seminal Plasma were 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. **(d)** Specificity of the assay; PC = phosphatidylcholine, PA = phosphatidic acid, SM = sphingomyelin, GPC = glycerophosphorylcholine.

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