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ab174092

PicoProbe Free Glycerol Assay Kit

Instructions for Use

For the sensitive and accurate measurement of glycerol in various samples

This product is for research use only and is not intended for diagnostic use.

Version: 2 Last Updated: 23 November 2015

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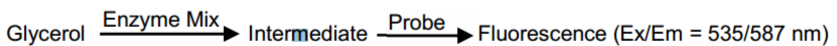
1. Overview

Glycerol is a central component for synthesis of all lipids; it acts as a backbone for triglycerides and phospholipids, which plays an important role for cell membrane’s structure. Due to its low toxicity, glycerol is widely used in pharmaceutical, food and cosmetic industries.

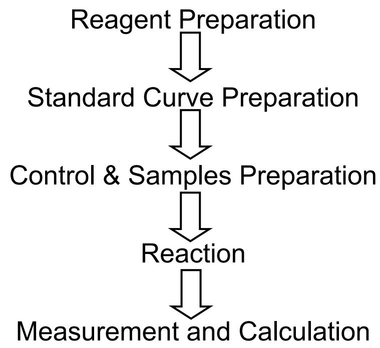
Abcam's PicoProbe Free Glycerol Assay Kit (ab174092) is simple, sensitive and easy to use. This assay is suitable for measuring trace amount of glycerol in samples, which contain reducing substances that may interfere with oxidase-based assays. In the assay, glycerol reacts with the enzyme mix to form an intermediate, which is subsequently oxidized with the production of fluorescence. The fluorescence intensity is directly proportional to the amount of glycerol.

This assay kit can detect glycerol amount less than 40 pmol.

Figure 1: Assay Procedure.



2. Protocol Summary



3. Kits Components

Item	Quantity
Glycerol Assay Buffer	25 mL
PicoProbe (in DMSO)	0.4 mL
Glycerol Enzyme Mix (Lyophilized)	1 vial
Glycerol Developer (Lyophilized)	1 vial
Glycerol Standard (100 mM)	0.2 mL

4. Storage and Stability

Upon arrival, store the kit at-20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Briefly centrifuge all small vials prior to opening.

5. Materials Required, Not Supplied

- 96-well black plate with flat bottom (for fluorometric reading)
- Multi-well fluorometric spectrophotometer (ELISA reader)
- Multi-channel pipette

6. Reagents Preparation

1. **Glycerol Assay Buffer:**
   Ready to use as supplied. Warm to room temperature before use. Store at -20°C.

2. **Glycerol Enzyme Mix**:
   Reconstitute with 220 µL Glycerol Assay Buffer, making sure the material is completely dissolved. Aliquot and store reconstituted mix at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Stable for 2 months at -20°C

3. **Glycerol Developer:**
   Reconstitute with 220 µL Glycerol Assay Buffer, making sure the material is completely dissolved. Aliquot and store reconstituted mix at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Stable for 2 months at -20°C

4. **PicoProbe (in DMSO):**
   Ready to use as supplied. Warm to room temperature before use to ensure DMSO is liquid. Store at -20°C after use.

7. Assay Protocol

1. Sample Preparation

a) Cells (starting material: 10^6 cells)

Harvest cells and spin down briefly and discard supernatant. Resuspend the cell pellet in 100 µL ice cold Glycerol Assay Buffer and put on ice. Homogenize with a Douncer homogenizer (10 – 15 passes) on ice, or by pipetting up and down using a smaller tip, until efficient lysis is confirmed by viewing the cells under the microscope. Centrifuge homogenate at 12000 rpm for 10 minutes at 4°C to remove cell debris and collect the supernatant.

Use the supernatant for your subsequent assays. Test different dilutions of the sample to ensure the readings will fall within the linear range of the standard curve.

Add 1 – 50 µL test sample to wells of a 96-well plate. If volume needed is <50 µL, bring it up to 50 µL with Assay Buffer.

b) Tissue(starting material: 10 mg)

Cut tissue in small pieces, add 100 µL ice cold Glycerol Assay Buffer and put on ice. Homogenize using a Douncer homogenizer (10 – 15 passes) on ice, until efficient lysis is confirmed, by viewing the cells under the microscope. Spin down the samples and collect the supernatant.

Use the supernatant for your subsequent assays. Test different dilutions of the sample to ensure the readings will fall within the linear range of the standard curve.

Add 1 – 50 µL test sample to wells of a 96-well plate. If volume needed is <50 µL, bring it up to 50 µL with Assay Buffer.

c) Liquid samples (plasma, serum, urine and other biological fluids):

Liquid samples can be tested directly.

Add 1 – 50 µL test sample to wells of a 96-well plate. If volume needed is <50 µL, bring it up to 50 µL with Assay Buffer.

NOTE: For unknown samples, we suggest testing several doses of your samples to ensure the readings are within the Standard Curve range.

d) BACKGROUND CONTROL

NADH in samples will generate background. For samples having high NADH levels, prepare parallel sample well(s) as background control.

2. Standard Curve Preparation:

a) Prepare a 1mM (1 nmol/µL) Glycerol Standard by adding 5 µL of the 100 mM Glycerol Standard (100 nmol/µL) into 495 µL of Glycerol Assay Buffer and mixing well by pipetting up and down.

b) Prepare a 60 μ (60pmol/μL) Glycerol Standard by adding 60 μL of 1mM Glycerol Standard to 940 μL Glycerol Assay Buffer. Mix well by pipetting up and down.

c) Using the 60 μM Glycerol standard, prepare standard curve dilution as follows, in a microplate or microcentrifuge tubes:

Glycerol 60μM amount (μl)	**Glycerol assay buffer (μL)**	**Amount in well**	**END CONCENTRATION GLYCEROL IN WELL**
0	150	50 μl	0 pmol/well
6	144	50 μl	120 pmol/well
12	138	50 μl	240 pmol/well
18	132	50 μl	360 pmol/well
24	126	50 μl	480 pmol/well
30	120	50 μl	600 pmol/well

Add 50 μl of each standard dilution into a well in a 96-well plate to set up standard.

Each dilution has enough amount of standard to set up 2 duplicates x 50 μl/well.

3. **Reaction Mix:**

Prepare Reaction Mix for each reaction:

	Reaction Mix	**Background Control Mix**
Glycerol Assay Buffer	43 µL	45 µL
PicoProbe	3 µL	3 µL
Glycerol Enzyme Mix	2 µL	----
Glycerol Developer	2 µL	2 µL

Mix enough reagents for the number of assays (samples and standards) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

	Reaction Mix
Glycerol Assay Buffer	43 µL x (Nb samples + Standards +1)
PicoProbe	3 µL x (Nb samples + Standards +1)
Glycerol Enzyme Mix	2 µL x (Nb samples + Standards +1)
Glycerol Developer	2 µL x (Nb samples + Standards +1)

We also recommend preparing enough **Background Control Mix** to set up duplicate readings for the NADH background control(s) using the same calculations.

4. Plate set up and Detection

a) Add 50 µL of standard and sample to wells.

b) Add 50 µL of the Reaction Mix to each well containing the Standards and test samples. Mix well.

c) Add 50 µL of Background Control Mix to 50 µL of background control well(s). Mix well.

d) Incubate the reaction for 60 min at room temperature, protected from light.

e) Measure fluorescence at Ex/ Em = 535/587 nm in a microplate reader.

8. Data Analysis

Calculations:

a) Correct background by substracting the value derived from the zero standard from all sample readings.

b) Plot the Glycerol Standard Curve.

c) If Background Control reading is significantly high, subtract the Background Control reading from sample reading. Apply corrected sample reading to the Glycerol Standard Curve to get B pmol of Glycerol in the sample wells.

Sample Glycerol concentration (C) = B/V x Dilution Factor = pmol/µL= nmol/mL = µM

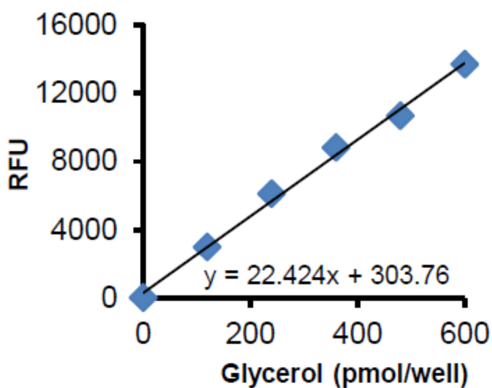
Where:

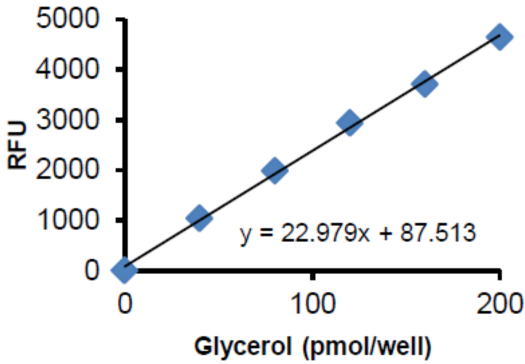
B = the amount of glycerol in the sample well (pmol).

V = volume of sample used in the reaction well (µL).

Glycerol molecular weight: 92.09 g/mol

Glycerol in sample can also be expressed in pmol/mg or mg/dL of sample.





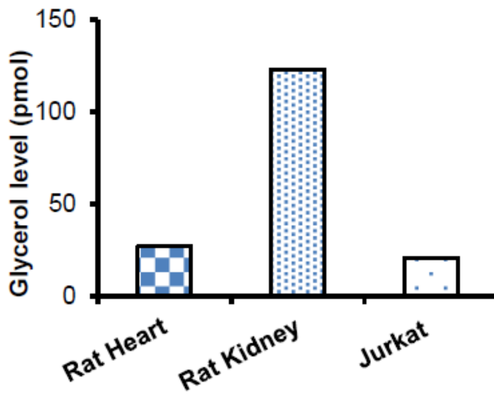


Figure 2. (a) & (b) Glycerol Standard curve; **(c)** Measurement of glycerol levels in rat heart (5 µg protein), rat kidney (5 µg) and Jurkat cell lysate (10 µg). Assays were performed according to kit protocol.

9. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region.

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