ab65337

Free Glycerol Assay kit (Colorimetric/Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Free Glycerol in various samples.

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

Version 8b, Last Updated 27 June 2025

Table of Contents

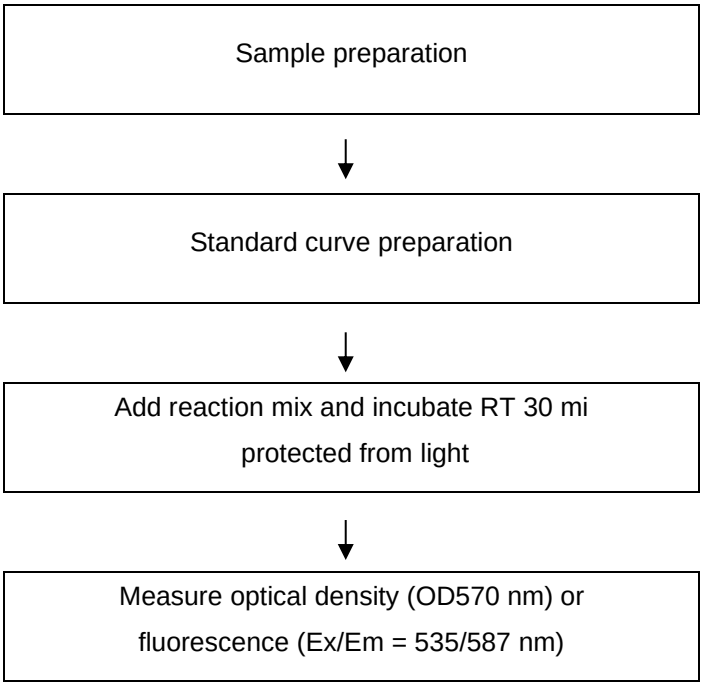
1. BACKGROUND

Free Glycerol Assay Kit (Colorimetric/Fluorometric) (ab65337) provides a sensitive, easy assay to measure free glycerol concentration in various samples. In the assay, glycerol is enzymatically oxidized to generate a product which reacts with the probe to generate color (λ= 570 nm) or fluorescence (Ex/Em = 535/587 nm). The assay can detect 50 pmol – 10 nmol (or ~1-10000 µM range) of glycerol in various samples.

Glycerol is the main component of triglycerides, the most important storage form of fat. It is an important metabolite in energy metabolism involved in both oxidation and synthetic processes. Moreover, it is an important component of the production of foods and beverages, solvents, pharmaceutical and cosmetic products, etc.

Under physiological conditions, triglycerides are hydrolyzed through lipolysis and release as glycerol and FFA (free fatty acid) in the blood. But unlike the FFA, glycerol cannot be reused by the adipose tissue. The quantification of circulating levels of glycerol and FFA is not only useful to evaluate lipolysis but there is also broad interest in quantification of glycerol for research and development.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer 5	25 mL	-20°C	-20°C
OxiRed™ Probe	0.2 mL	-20°C	-20°C
Enzyme Mix VI	1 vial	-20°C	-20°C
Glycerol Standard	200 µL	-20°C	-20°C

PLEASE NOTE: Assay Buffer 5 was previously labelled as Assay Buffer V and Glycerol Assay Buffer, and OxiRed™ Probe as OxiRed Probe and Glycerol Probe (in DMSO, anhydrous). The composition has not changed.

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric or fluorescent microplate reader – equipped with filter for OD570 nm or Ex/Em = 535/587 nm (respectively)
- 96 well plate: clear plates for colorimetric assay; black plates (clear bottoms) for fluorometric assay
- Dounce homogenizer or pestle (if using tissue)
- Orbital shaker

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer 5:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C protected from light.

9.2 **OxiRed™ Probe:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.** Store at -20°C protected from light. Aliquot OxiRed™ Probe so that you have enough volume to perform the desired number of assays. Once the OxiRed™ Probe is thawed, use with two months.

9.3 **Enzyme Mix VI:**

Reconstitute in 200 µL Assay Buffer 5. Aliquot Enzyme Mix VI/enzyme so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice during the assay.

9.4 **Glycerol Standard:**

Ready to use as supplied. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice during the assay.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 **For the colorimetric assay:**

10.1.1 Prepare a 1mM Glycerol standard by diluting 10 µL of the provided Glycerol Standard with 990 µL of Assay Buffer 5.

10.1.2 Using 1 mM Glycerol standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer 5 (µL)	Final volume standard in well (µL)	End [Glycerol] in well
1	0	150	50	0 nmol/well
2	6	144	50	2 nmol/well
3	12	138	50	4 nmol/well
4	18	132	50	6 nmol/well
5	24	126	50	8 nmol/well
6	30	120	50	10 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

10.2 **For the fluorometric assay:**

10.1.1 Prepare a 1 mM Glycerol standard by diluting 5 µL of the provided Glycerol Standard (10 mM) with 495 µL of Assay Buffer 5.

10.1.2 Prepare 100 µL of 0.1 mM Standard by diluting 10 µL of 1 mM Glycerol Standard with 90 µL of Assay Buffer 5.

10.1.3 Using 0.1 mM Glycerol standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer 5 (µL)	Final volume standard in well (µL)	End [Glycerol] in well
1	0	150	50	0 nmol/well
2	6	144	50	0.2 nmol/well
3	12	138	50	0.4 nmol/well
4	18	132	50	0.6 nmol/well
5	24	126	50	0.8 nmol/well
6	30	120	50	1.0 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

NOTE: *If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.*

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
- Certain cell or tissue samples may need to be treated with Carrez Clarification Reagent Kit (ab202373).

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Spin down and briefly and discard supernatant.

11.1.4 Resuspend cells in 500 µL (or 4x volumes) of Assay Buffer 5 and put on ice.

11.1.5 Homogenize using a Douncer homogenizer (10-15 passes) on ice.

11.1.6 Spin down the samples and collect the supernatant.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 – 100 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Add 0.5 – 1mL (or 4x volumes) of Assay Buffer 5 and put on ice.

11.2.4 Homogenize using a Dounce homogenizer (10-15 passes) on ice.

11.2.5 Centrifuge samples for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect the supernatant.

11.3 **Serum and urine samples**

Treat serum samples with Carrez Clarification Reagent Kit (ab202373) to remove anti-oxidants. Add 10 µL supernatant to each well and adjust to 50 µL with Assay Buffer 5. Urine samples can be assayed directly or after dilution in Assay Buffer 5.

NOTE: *We suggest using different dilutions of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL Standard dilutions.
- Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 5).

12.2 **Reaction Mix:**

Prepare 50 µL of Reaction Mix for each reaction:

Component	Colorimetric Reaction Mix (µL)	Fluorometric Reaction Mix (µL)
Assay Buffer 5	**46**	**47.6**
OxiRed™ Probe*	**2**	**0.4**
Enzyme Mix VI	**2**	**2**

NOTE: *For fluorometric reading, using 0.4µL/well of the OxiRed™ Probe decreases the background readings, therefore increasing detection sensitivity.*

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

X µL component x (Number samples + standards +1).

12.3 Add 50 µL of Reaction Mix into each standard and sample well.

12.4 Mix and incubate at room temperature for 30 minutes protected from light.

12.5 Measure output on a microplate reader.

- Colorimetric assay: measure OD570 nm.
- Fluorometric assay: measure Ex/Em = 535/587 nm

The reaction is stable for 2 hours.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of Glycerol.

13.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.5 Extrapolate sample readings from the standard curve plotted using the following equation:

$$Ga = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

13.6 Concentration of glycerol in the test samples is calculated as:

$$Glycerol\ concentration = \left(\frac{Ga}{Sv}\right) * D$$

Where:

Ga = Glycerol amount from standard curve (nmol).

Sv = sample volume added in sample wells (µL).

D = Sample dilution factor.

Glycerol molecular weight: 92.09 g/mol.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

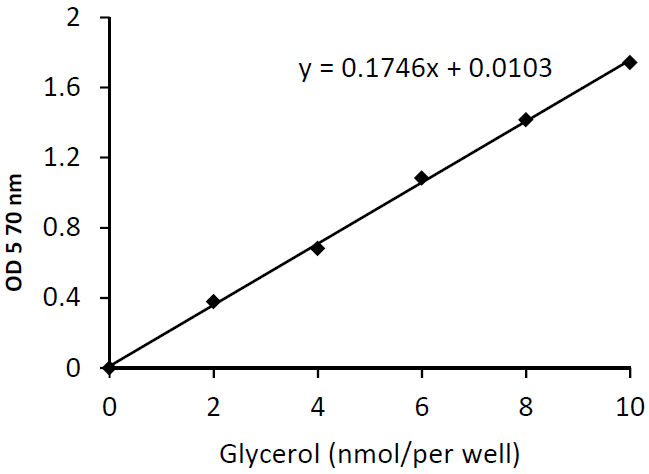


Figure 1: Typical Glycerol calibration curve performed using colorimetric reading.

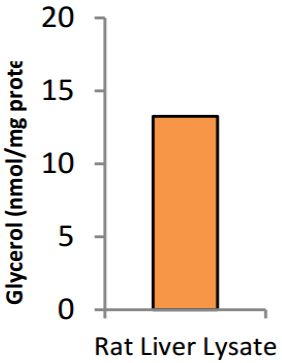


Figure 2: Measurement of free Glycerol in rat liver lysate (500 µg). Assay was performed following the kit protocol.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing this assay for the first time.*

- Solubilize Enzyme Mix VI, thaw OxiRed™ Probe and Assay Buffer 5 (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve for your detection method of choice (colorimetric or fluorometric).
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL) and samples (50 µL).
- Prepare Glycerol Reaction Mix (Number samples + standards + 1).

Component	Colorimetric Reaction Mix (µL)	Fluorometric Reaction Mix (µL)
Assay Buffer 5	**46**	**47.6**
OxiRed™ Probe	**2**	**0.4**
Enzyme Mix VI	**2**	**2**

- Add 50 µL of Glycerol Reaction Mix to the standard and sample wells.
- Incubate plate at RT for 30 minutes protected from light.
- Measure plate at OD570 nm for colorimetric assay or Ex/Em= 535/587 nm for fluorometric assay.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of inappropriate plate for reader	Colorimetry: Clear plates Fluorescence: Black plates (clear bottom)
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer (increase number of strokes); observe for lysis under microscope
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so as to be in the linear range

17. FAQs

Should medium to be assayed by this kit be stored at -20°C or 4°C?

We recommend storing the medium at -20°C or preferably at -80°C for best results. If the assay will be done in multiple batches, aliquots can be stored to minimize freezing-thawing.

Can glycerine be used in this assay?

Yes, Glycerine is same as glycerol and can be used with this kit.

What is the detection range for the colorimetric assay?

The colorimetric assay shows a linear standard curve in the micromolar (mM) concentration range of glycerol.

Can this kit be used with bacterial/yeast samples?

All Abcam kits are optimized with mammalian samples but can work with many other species including yeast. One consideration is that yeast cells have cell walls and hence might need specific reagents to break the cell wall in yeast cells and some bacterial cells.

Can I use the same method of samples preparation in the protocol of Triglyceride Quantification Kit (ab65336) to prepare the samples to use this Free Glycerol Assay Kit to measure intracellular glycerol?

This will not work. For the triglyceride assay (ab65336) lipid extracts are prepared from the cells while for the Free glycerol assay cell lysate/tissue homogenate is used.

18. **INTERFERENCES**

19. NOTES

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)