EZ-Free Glycerol Assay Kit

Metabolism assay kit

(Colorimetric/Fluorometric)

Cat. No. DG-FGC100

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

DoGenBio Co., Ltd.

▪ Product Description

Glycerol ($C_3H_8O_3$) is the main component of triglyceride and the main storage form of fat. In vivo, it plays a role as a key ingredient in energy metabolism processes including oxidation and biosynthesis, and is widely used in industry, including beverages, solvents, pharmaceuticals, and cosmetics. Under physiological conditions, triglyceride is hydrolyzed into glycerol and free fatty acid through the lipolysis process, but unlike free fatty acid, glycerol is not reused in adipose tissue.

EZ-Free Glycerol Assay Kit provides a sensitive and easy analysis method to measure the concentration of free glycerol in a variety of samples. In the assay, Glycerol allows the enzymatically oxidized product to react with the probe to measure absorbance (λ = 570 nm) and fluorescence (Ex/Em = 535/590 nm).

▪ Contents and Storage Conditions

Component	100 assay	Cap Cord	Storage (reconstituted)
Glycerol Assay buffer	25 ㎖	-	-20℃, 2 months
Glycerol Enzyme mix (Lyophilized)	1 vial	Red	-20℃, 2 months
Glycerol Probe	200 ㎕	Yellow	-20℃, 2 months
Glycerol Standard (100 mM)	100 ㎕	Green	-20℃, 2 months

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

* In terms of the number of tests that can be performed with this product, 100 assays means that it provides reagents that can process 100 wells based on 1 well of a 96 well plate. Among these, considering standard, blank, duplication processing per sample, etc., the actual number of samples that can be tested is in the range of 20 to 40 samples. Review the product instructions carefully and determine the number of kits required considering the characteristics of the sample you wish to test.

▪ Preparation of Reagent

Component	Preparation	Storage and Stability
Glycerol Enzyme mix (Lyophilized)	Add 220 ㎕ Assay Buffer and mix well using a pipette.	The mixed solution is stable for 2 months at -20℃.
Glycerol Probe	Use after sufficiently dissolving at room temperature.	Glycerol Probe is stable for 2 months at -20℃.
Glycerol Standard	Use after sufficiently dissolving at room temperature.	The remaining solution can be stored at -20℃, but use it within 2 months.

* Assay buffer is used after sufficiently warming up to room temperature before experiment.
* When using a cold buffer, enzyme activity may be inhibited, affecting measurement results.

▪ General Protocol

1. Sample preparation

After adding 2-50 ㎕ of the prepared sample into a 96 well plate, adjust the final volume to 50 ㎕ with assay buffer. (n≥2)

1) Serum sample
Using Carrez reagent (purchased individually), antioxidant substances from proteins and fats are removed in advance.

2) Cell or Tissue

① Prepare ~ 2 x 10^6 cells or ~ 10 mg tissue sample.
② Wash the sample using PBS.
③ Add 500 ㎕ of Glycerol Assay buffer solution to the sample and homogenize on ice.
④ Centrifuge at 10,000xg at 4℃ for 10 minutes and use the supernatant for analysis.
* For specific cells and tissues, Carrez reagent (purchased separately) may be required.

3) Urine sample : Analysis is possible without preprocessing.

4) For unknown samples or samples being measured for the first time, ensure that the measured values are within the standard curve. We recommend using it after preliminary testing.

5) For samples with high background, prepare the same amount of sample used for measurement as a background control.

2. Standard preparation

1) Colorimetric method

Mix 10 ㎕ of 100 mM Glycerol standard solution and 990 ㎕ of distilled water. Make 1mM standard solution. Add 0, 2, 4, 6, 8, and 10 ㎕ into each 96 well plate and then adjust the final volume to 50 ㎕ with assay buffer. A standard set of 0, 2, 4, 6, 8, and 10 nmol/well is added to each plate.

Standard No.	Volume of 1mM Glycerol Standard	Volume of Assay buffer	Final standard volume in well	Final standard Glycerol Conc. (nmol/well)
1	0 ㎕	50 ㎕	50 ㎕	0
2	2 ㎕	48 ㎕	50 ㎕	2
3	4 ㎕	46 ㎕	50 ㎕	4
4	6 ㎕	44 ㎕	50 ㎕	6
5	8 ㎕	42 ㎕	50 ㎕	8
6	10 ㎕	40 ㎕	50 ㎕	10

* For accurate measurement, it is recommended to prepare and conduct experiments with more than two replicates each of standards and samples.

* Standard recommends measurement during each experiment.

2) Fluorometric method

Mix 10 ㎕ of 100 mM Glycerol standard solution and 990 ㎕ of distilled water. Make 1mM standard solution. Distilled with 100 µl of 1 mM Glycerol standard solution Mix 900 ㎕ of water to make a 0.1 mM standard solution. Add 0, 2, 4, 6, 8, and 10 ㎕ into each 96 well plate and then adjust the final volume to 50 ㎕ with assay buffer. A standard set of 0, 0.2, 0.4, 0.6, 0.8, and 1 nmol/well is added to each plate.

Standard No.	Volume of 0.1mM Glycerol Standard	Volume of Assay buffer	Final standard volume in well	Final standard Glycerol Conc. (nmol/well)
1	0 ㎕	50 ㎕	50 ㎕	0
2	2 ㎕	48 ㎕	50 ㎕	0.2
3	4 ㎕	46 ㎕	50 ㎕	0.4
4	6 ㎕	44 ㎕	50 ㎕	0.6
5	8 ㎕	42 ㎕	50 ㎕	0.8
6	10 ㎕	40 ㎕	50 ㎕	1

* For accurate measurement, it is recommended to prepare and conduct experiments with more than two replicates each of standards and samples.

* Standard recommends measurement during each experiment.

3. Reaction mixture preparation

This is the volume based on one well assay. Calculate the assay volume to be used in the experiment and prepare the reaction mix considering the loss volume.

1) Colorimetric method

Components	Reaction mixture	Background mixture
Glycerol Assay buffer	46 ㎕	48 ㎕
Glycerol Enzyme mix	2 ㎕	-
Glycerol Probe	2 ㎕	2 ㎕
Total	50 ㎕	50 ㎕

2) Fluorometric method

Components	Reaction mixture	Background mixture
Glycerol Assay buffer	47.6 ㎕	49.6 ㎕
Glycerol Enzyme mix	2 ㎕	-
Glycerol Probe	0.4 ㎕	0.4 ㎕
Total	50 ㎕	50 ㎕

4. Add 50㎕ of the reaction mixture to each well containing the Glycerol standard and sample using a multi pipette, then mix well.

* If you have prepared a background control, add the background mixture to the background control well.

5. After incubation the plate at room temperature blocked from light for 30 minutes, shake gently and measure using a microplate reader.

1) Colorimetric : 570 nm

2) Fluorometric: (Excitation/Emission): 535 nm / 595 nm

▪ Calculation

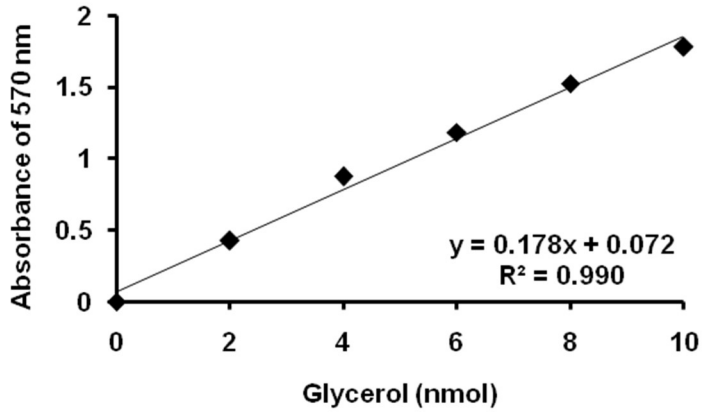
1. Standard 1 value (blank) is subtracted from all measured values.
2. Subtract the sample background measurement value from the sample measurement value.
3. Determine the standard curve using ATP standard measurements.
4. Calculate the amount of Glycerol in the sample by substituting the sample measurement value into the standard curve.

* If background control is set, the value obtained by subtracting the background control measurement value from the sample measurement value is Substitute to calculate the amount of Glycerol.

5. Based on the amount of glycerol calculated in 4, calculate the concentration of glycerol in the sample using the following equation.

Glycerol concentration in the sample (C) = B/V x D (nmol/㎕ or mM)

B : ATP amount in measuring well(nmol)
V : Amount of sample added into the well(㎕)
D : Sample dilution factor (if diluted 2-fold, calculate as x2, not x 1/2)



Glycerol standard curve. Assay was performed following the kit protocol.

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▪ Related Product

	Products	Catalog No.	Assay
Oxidative Stress Assay Kit	EZ-Superoxide Dismutase (SOD) Assay Kit (Colorimetric)	DG-SOD400	400 Assay
	EZ-Glutathione Assay Kit (Colorimetric)	DG-GLU200	200 Assay
	EZ-Catalase Assay Kit (Fluorometric/Colorimetric)	DG-CAT400	400 Assay
	EZ-Hydrogen peroxide/Peroxidase Assay Kit (Fluorometric/Colorimetric)	DG-PER500	500 Assay
	EZ-Lipid Peroxidation (TBARS) Assay Kit (Colorimetric)	DG-TBA200	200 Assay
	EZ-Total Antioxidant Capacity (TAC) Assay Kit (Colorimetric)	DG-TAC200	200 Assay
	EZ-DPPH Antioxidant Assay Kit (Colorimetric)	DG-DPH400	400 Assay
	EZ-ABTS Antioxidant Assay Kit (Colorimetric)	DG-ABT400	400 Assay
	EZ-Glutathione Peroxidase Assay Kit (Colorimetric)	DG-GPX100	100 Assay
Metabolism Assay Kit	EZ-Lactate Assay Kit (Colorimetric)	DG-LAC100	100 Assay
	EZ-Acetylcholinesterase Assay Kit (Colorimetric)	DG-ACE100	100 Assay
	EZ-Ascorbic Acid Assay Kit (Colorimetric)	DG-ASC100	100 Assay
	EZ-ATP Assay Kit (Fluorometric/Colorimetric)	DG-ATP100	100 Assay
	EZ-Free Fatty Acid Assay Kit (Fluorometric/Colorimetric)	DG-FFA100	100 Assay
	EZ-Free Glycerol Assay Kit (Fluorometric/Colorimetric)	DG-FGC100	100 Assay
	EZ-Glucose Assay Kit (Fluorometric/Colorimetric)	DG-GCS100	100 Assay
	EZ-HDL, LDL/VDL Assay Kit (Fluorometric/Colorimetric)	DG-CHO100	100 Assay
	EZ-Total Cholesterol Assay Kit (Fluorometric/Colorimetric)	DG-TSC100	100 Assay
	EZ-Triglyceride Quantification Assay Kit (Fluorometric/Colorimetric)	DG-TGC100	100 Assay
	EZ-Nitric Oxide Assay kit (Colorimetric)	DG-NO500	500 Assay
	EZ-Total Collagen Assay Kit (Colorimetric)	DG-COL100	100 Assay
	EZ-Ethanol Assay Kit (Colorimetric)	DG-ETH100	100 Assay