TECHNICAL MANUAL

Glucose Uptake Fluorometric Assay Kit

- **SKU CODE:** MAES0012
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Cell pretreatment
- Glucose uptake process
- Standard and sample preparation
- Measure the fluorescence value
- Calculation

2. Intended use

This kit can be used to measure glucose uptake content in cell samples.

3. Detection principle

2-Deoxyglucose (2-DG) is taken up by cells and converted to 2-DG-6-phosphate, which is catalyzed by glucose dehydrogenase to produce 6-phosphogluconate. Meanwhile, NADP+ is converted to NADPH. The generated NADPH converts the probe into fluorescent substances under the action of diaphorase. The glucose uptake can be calculated by measuring the fluorescence intensity at the excitation wavelength of 530 nm and the emission wavelength of 590 nm.

4. Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Acid Reagent	10 mL x 1 vial	55 mL x 1 vial	-20 °C, 12 months
Reagent 2	Alkali Reagent	10 mL x 1 vial	55 mL x 1 vial	-20 °C, 12 months
Reagent 3	Chromogenic Agent	25 mL x 1 vial	45 mL x 3 vials	-20 °C, 12 months, protect from light
Reagent 4	Enzyme Reagent	Powder x 2 vials	Powder x 10 vials	-20 °C, 12 months
Reagent 5	10 mmol/L 2-DG	1.5 mL x 1 vial	8 mL x 1 vial	-20 °C, 12 months
Reagent 6	0.3 nmol/μL Standard	2 mL x 1 vial	5 mL x 2 vials	-20 °C, 12 months

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 7	KRPH Buffer Solution	55 mL x 1 vial	60 mL x 5 vials	-20 °C, 12 months
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=530 nm/590 nm), micropipettor, incubator, water bath

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of enzyme working solution:** Dissolve one vial of enzyme reagent with 10 mL of chromogenic agent, mix well to dissolve. Store at 2-8 °C for 3 days protected from light.
3. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 0.3 nmol/μL standard with double distilled water to serial concentrations. The recommended dilution gradient is as follows: 0, 0.06, 0.12, 0.15, 0.18, 0.21, 0.24, 0.3 nmol/μL.

7. Operating steps

1. **Cell pretreatment:** Seed cells at a density of 20,000-50,000 cells per well in a 96-well plate. The cells can be cultured and treated according to experimental needs.
2. **Uptake process:** Starve the cells overnight in serum-free cell medium (starvation time can be 6-8 hours; monitor cell state), then discard the medium. Wash cells twice with 200 μL of KRPH solution (including 2% BSA), add 100 μL of KRPH solution to the control well and sample well (including 2% BSA), then add 10 μL of 10 mmol/L 2-DG to the sample well in the cell culture plate, and add 10 μL of KRPH solution to the control well. Incubate at 37 °C for 30 min.

3. **Cell lysis and extraction:** Wash cells 3 times with 100 µL of KRPH solution, add 50 µL of acid reagent and stand at room temperature for 10 min, then add 50 µL alkali reagent.
4. **Sample and standard preparation:** Standard well: Take 30 µL of standards with different concentrations into the corresponding fluorescence standard wells. Sample well: Take 30 µL from sample well into the corresponding fluorescence wells. Control well: Take 30 µL from control well into the corresponding fluorescence wells.
5. **Enzymatic reaction:** Add 170 µL of enzyme reagent working solution into each well.
6. **Incubation:** Incubate at 37 °C for 30 min.
7. **Measurement:** Measure the fluorescence intensity of each well at the excitation wavelength of 530 nm and the emission wavelength of 590 nm.

8. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #1) from all standard readings. This is the absolute fluorescence value.

3. Plot the standard curve using absolute fluorescence value of standards and corresponding concentrations as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

Glucose uptake content (nmol/µL) = $(F_2 - F_1 - b) \div a$

[Note]

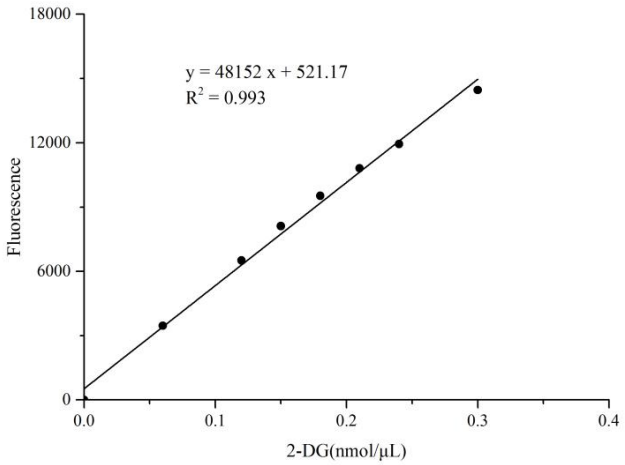
F_1: The fluorescence intensity of control well.

F_2: The fluorescence intensity of sample well.

9. Appendix I Performance Characteristics

Standard curve:

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g., operator, pipetting technique, or temperature effects), the standard curve and data are provided below for reference only:



10. Appendix II Example Analysis

Example analysis:

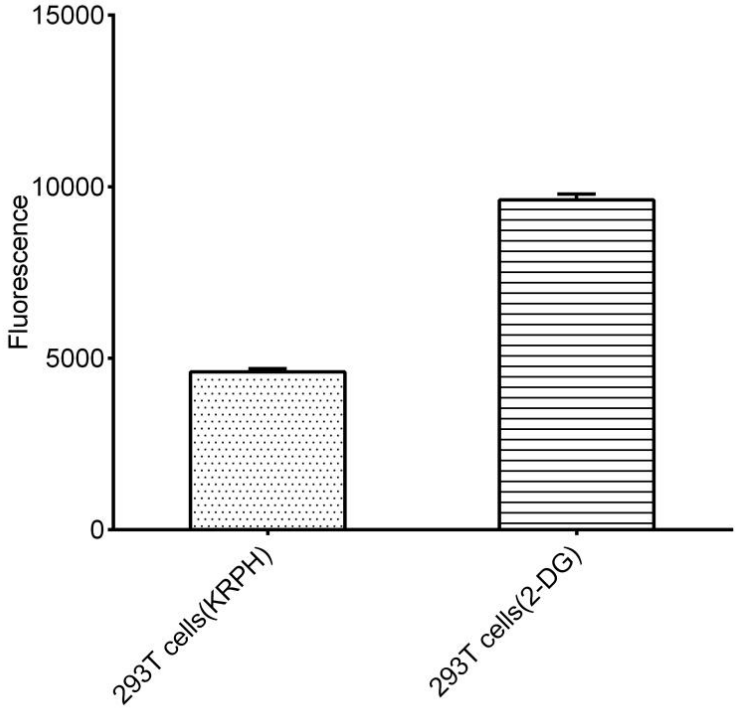
For 293T cells (0.7 x 10^6 cells), carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 48152x + 521.17

The fluorescence value of the sample (F_2) is 9614, the fluorescence value of the control (F_1) is 4603, and the calculation result is:

Glucose uptake content (nmol/µL) = (9614 - 4603 - 521.17) ÷ 48152 = 0.093 nmol/µL

Detect 293T cells according to the protocol, the result is as follows:



11. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.