Technical Manual

Glucose Uptake Fluorometric Assay Kit

- Catalogue Code: MAES0012
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Fluorimetric method

Specification:

96T

Range:

0.02-0.3 nmol/μL

Sensitivity:

0.02 nmol/μL

Storage:

-20°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Glucose uptake is an important biological process for studying cell signaling and glucose metabolism. Among many different methods available for measuring glucose uptake, 2-deoxyglucose (2-DG) has been widely used because of its structural similarity to glucose. As with glucose, 2-DG can be taken up by glucose transporters, and metabolized to 2-DG-6-phosphate (2-DG-6P). 2-DG-6P, however, cannot be further metabolized, and thus accumulates in the cells. The accumulated 2-DG-6P is proportional to 2-DG (or glucose) uptake by cells.

3. Intended Use

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

4. Detection Principle

2-DG is up-taken by the cells, converted to 2-DG-6P, which is catalyzed by glucose dehydrogenase to produce 6PDG. Meanwhile, $NADP^+$ is converted to NADPH. The generated NADPH converts the probe into fluorescent substances under the action of myocardial yellow transferase. The glucose uptake can be calculated by measuring the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 590 nm.

5. Kit components & storage

Item	Specification	Storage
Acid Reagent	10 mL × 1 vial	-20°C, 6 months
Alkali Reagent	10 mL × 1 vial	-20°C, 6 months
Chromogenic Agent	25 mL × 1 vial	-20°C, 6 months, avoid direct sunlight
Enzyme Reagent	Lyophilized × 2 vials	-20°C, 6 months
2-DG (10 mmol/L)	1.5 mL × 1 vial	-20°C, 6 months
Standard (0.3 nmol/μL)	2 mL × 1 vial	-20°C, 6 months
Black Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Fluorescence microplate reader (Ex/Em=530 nm/590 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- 5 mM KRPH solution (20 mM Hepes, 5 mM KH_2PO_4, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 136 mM NaCl, 4.7 mM KCl, 2% BSA, pH 7.4)

6. Assay Notes:

For other cell types, optimal incubation time may vary from this conditions.

7. Reagent Preparation

1. Bring all reagent to room temperature before use.

2. Preparation of **working solution:** Dissolve a vial of enzyme reagent with 10 mL of chromogenic agent and mix fully. The prepared solution can be stored at 2-8°C for 3 days with avoid direct sunlight.

8. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: Ex/Em=535 nm/590 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1′	S9	S9′	S17	S17′	S25	S25′	S33	S33′
B	B	B	S2	S2′	S10	S10′	S18	S18′	S26	S26′	S34	S34′
C	C	C	S3	S3′	S11	S11′	S19	S19′	S27	S27′	S35	S35′
D	D	D	S4	S4′	S12	S12′	S20	S20′	S28	S28′	S36	S36′
E	E	E	S5	S5′	S13	S13′	S21	S21′	S29	S29′	S37	S37′
F	F	F	S6	S6′	S14	S14′	S22	S22′	S30	S30′	S38	S38′
G	G	G	S7	S7′	S15	S15′	S23	S23′	S31	S31′	S39	S39′
H	H	H	S8	S8′	S16	S16′	S24	S24′	S32	S32′	S40	S40′

Note: A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells.

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9. Operation Steps

The preparation of standard curve

Dilute standard (0.3 nmol/μL) with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.06, 0.12, 0.15, 0.18, 0.21, 0.24, 0.3 nmol/μL.

Cell pretreatment

Dilute standard (0.3 nmol/μL) with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.06, 0.12, 0.15, 0.18, 0.21, 0.24, 0.3 nmol/μL.

Uptake process

1. Starve the cells overnight in serum-free cell medium (starved cells), 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.

2. Wash cells for 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.

3. **Standard well:** Take 30 μL of standards with different concentrations into the corresponding fluorescence standard wells.
   Sample well: Take 30 μL from sample well of step 2 into the corresponding fluorescence wells.
   Control well: Take 30 μL from control well of step 2 into the corresponding fluorescence wells.

4. Add 170 μL of working solution into each well.

5. Incubate at 37°C for 30 min.

6. Measure the fluorescence intensity of each well at the excitation wavelength of 530 nm and the emission wavelength of 590 nm.

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10. Calculations

Plot the standard curve by using fluorescence value (F) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y = ax + b.

Glucose uptake content (nmol/μL)

$= (F_2 - F_1 - b) \div a$

y: The absolute fluorescence value of standard, $F_{Standard} – F_{Blank}$ (F_{Blank} is the F value when the standard concentration is 0)

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve

F_1: The fluorescence intensity of control well

F_2: The fluorescence intensity of sample well

11. Performance Characteristics

Detection Range	0.02-0.5 nmol/μL
Sensitivity	0.02 nmol/μL

Analysis

For 293T cells (0.7×10^6 cells), carry the assay according to the operation table.

The results are as follows:

standard curve: y = 48152 x + 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

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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