PRODUCT INFORMATION & MANUAL

Glucose Uptake Assay Kit (Fluorometric)

NBP3-25776

For research use only.
Not for diagnostic or therapeutic procedures.

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Glucose Uptake Assay Kit (Fluorometric)

Catalog No: NBP3-25776

Method: Fluorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Measuring instrument: Fluorescence Microplate Reader

Sensitivity: 0.02 nmol/μL

Detection range: 0.02-0.3 nmol/μL

▲ This kit is for research use only.
▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲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.

▲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 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.

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Acid Reagent	10 mL × 1 vial	-20℃ , 12 months
Reagent 2	Alkali Reagent	10 mL × 1 vial	-20℃ , 12 months
Reagent 3	Chromogenic Agent	25 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 4	Enzyme Reagent	Powder × 2 vials	-20℃ , 12 months
Reagent 5	10 mmol/L 2-DG	1.5 mL × 1 vial	-20℃ , 12 months
Reagent 6	0.3 nmol/μL Standard	2 mL × 1 vial	-20℃ , 12 months
Reagent 7	KRPH Buffer Solution	55 mL × 1 vial	-20℃ , 12 months
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Fluorescence microplate reader (Ex/Em=530 nm/590 nm), Micropipettor, Incubator, Water bath

Reagents

Double distilled water

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

⚠ Precautions

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

▲ The key points of the assay

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

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.
2. The preparation of reagent 4 working solution:

   Dissolve a vial of reagent 4 with 10 mL of reagent 3 and mix fully. The prepared solution can be stored at 2-8℃ for 3 days with shading light.

Assay protocol

▲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.

▲Detailed operation steps

The preparation of standard curve

Dilute 0.3 nmol/μL standard 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. Reference is as follows:

Number	Standard concentrations (nmol/μL)	0.3 nmol/μL standard (μL)	Double distilled water (μL)
A	0	0	200
B	0.06	40	160
C	0.12	80	120
D	0.15	100	100
E	0.18	120	80
F	0.21	140	60
G	0.24	160	40
H	0.3	200	0

Cell pretreatment

The cells were seeded at a density of ~2000 cells per well in a 96 well plate. The cells can be cultured and treated according to the experimental needs.

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 reagent 1 and stand at room temperature for 10 min, then add 50 μL reagent 2.
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 into the corresponding fluorescence wells.
   Control well: Take 30 μL from control well into the corresponding fluorescence wells.
4) Add 170 μL of reagent 4 working solution into each well.
5) Incubate at 37℃ 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.

▲Calculation

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 fluorescence value of sample.

The standard curve is: y= ax + b.

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

Note:

y: $F_{Standard} - F_{Blank}$. (F_{Blank} is the fluorescence 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.

Appendix I Data

▲Example 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=0.093nmol/μL