（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F041

Specification：96T(40 samples)/500Assays(242 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=530 nm/590 nm)

Detection range：0.02-0.3 nmol/μL

Elabscience® Glucose Uptake Fluorometric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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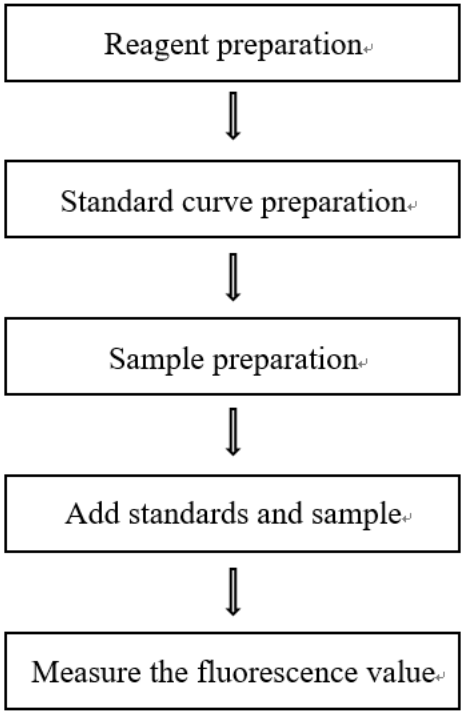
Assay summary



Intended use

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

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	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Acid Reagent	10 mL × 1 vial	55 mL × 1 vial	-20℃, 12 months
Reagent 2	Alkali Reagent	10 mL × 1 vial	55 mL × 1 vial	-20℃, 12 months
Reagent 3	Chromogenic Agent	25 mL × 1 vial	45 mL × 3 vials	-20℃, 12 months shading light
Reagent 4	Enzyme Reagent	Powder × 2 vials	Powder × 10 vials	-20℃, 12 months
Reagent 5	10 mmol/L 2-DG	1.5 mL × 1 vial	8 mL × 1 vial	-20℃, 12 months
Reagent 6	0.3 nmol/μL Standard	2 mL × 1 vial	5 mL × 2 vials	-20℃, 12 months
Reagent 7	KRPH Buffer Solution	55 mL × 1 vial	60 mL × 5 vials	-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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The 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℃ for 3 days protected from light.

③ The 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 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:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (nmol/μL)	**0**	**0.06**	**0.12**	**0.15**	**0.18**	**0.21**	**0.24**	**0.3**
0.3 nmol/μL standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

Operating steps

1. 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.

2. 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 ℃ 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 into the corresponding fluorescence wells.

Control well: Take 30 μL from control well of into the corresponding fluorescence wells.

④ Add 170 μL of enzyme reagent 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

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

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

[Note]

F_1: The fluorescence intensity of control well.

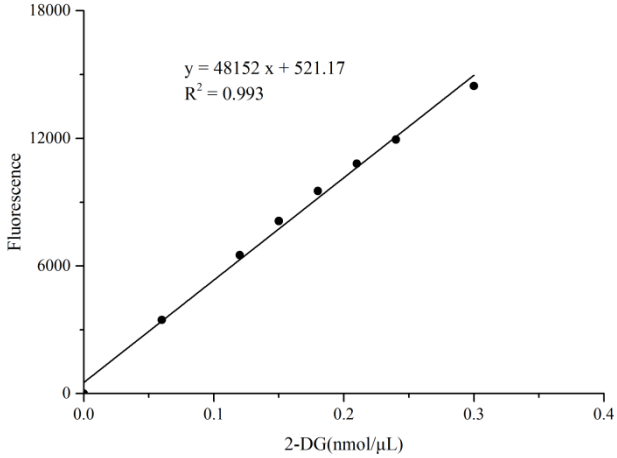
F_2: The fluorescence intensity of sample well.

Appendix I Performance Characteristics

1. 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), so the standard curve and data are provided as below for reference only:

Concentration (nmol/μL)	**0**	**0.06**	**0.12**	**0.15**	**0.18**	**0.21**	**0.24**	**0.3**
Fluorescence value	5176	8677	11778	13332	14730	16049	17242	19704
	5211	8643	11635	13276	14717	15968	17037	19603
Average fluorescence value	5194	8660	11707	13304	14724	16009	17140	19654
Absoluted fluorescence value	0	3467	6513	8111	9530	10815	11946	14460



Appendix II Example Analysis

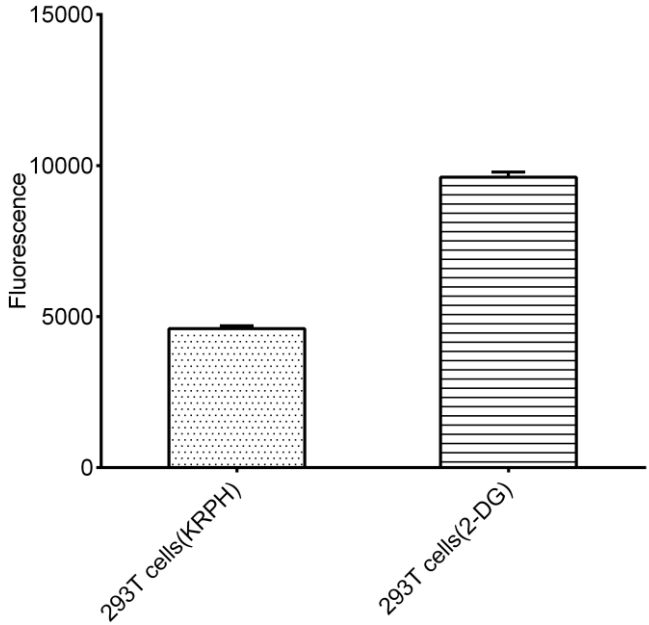
Example analysis：

For 293T cells (0.7×10^6 cells), carry the assay according to the operation steps. 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 (F1) 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：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.