# Elabscience ${ }^{\circledR}$ Glucose Uptake Fluorometric Assay Kit 

Catalog No: E-BC-F041

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

> Measuring instrument: Fluorescence Microplate Reader $$
(E x / E m=530 \mathrm{~nm} / 590 \mathrm{~nm})
$$

## Detection range: $\mathbf{0 . 0 2 - 0 . 3 ~ n m o l} / \mu \mathrm{L}$

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:

Phone: 240-252-7368(USA)
Fax: 240-252-7376(USA)
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Website: www.elabscience.com

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

## Table of contents

Assay summary ..... 3
Intended use ..... 4
Detection principle ..... 4
Kit components \& storage ..... 4
Materials prepared by users ..... 5
Reagent preparation ..... 5
Operating steps ..... 6
Calculation ..... 7
Appendix I Performance Characteristics ..... 8
Appendix П Example Analysis ..... 9
Statement ..... 10


## 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 <br> $\mathbf{( 9 6 ~ T )}$ | Size 2 <br> $\mathbf{( 5 0 0 ~ A s s a y s )}$ | Storage |
| :---: | :---: | :---: | :---: | :---: |
| Reagent 1 | Acid Reagent | $10 \mathrm{~mL} \times 1 \mathrm{vial}$ | $55 \mathrm{~mL} \times 1 \mathrm{vial}$ | $-20^{\circ} \mathrm{C}, 12 \mathrm{months}$ |
| Reagent 2 | Alkali Reagent | $10 \mathrm{~mL} \times 1 \mathrm{vial}$ | $55 \mathrm{~mL} \times 1$ vial | $-20^{\circ} \mathrm{C}, 12 \mathrm{months}$ |
| Reagent 3 | Chromogenic Agent | $25 \mathrm{~mL} \times 1 \mathrm{vial}$ | $45 \mathrm{~mL} \times 3$ vials | $-20^{\circ} \mathrm{C}, 12$ months <br> shading light |
| Reagent 4 | Enzyme Reagent | Powder $\times 2$ vials | Powder $\times 10$ vials | $-20^{\circ} \mathrm{C}, 12$ months |
| Reagent 5 | $10 \mathrm{mmol} / \mathrm{L} 2-$ DG | $1.5 \mathrm{~mL} \times 1$ vial | $8 \mathrm{~mL} \times 1$ vial | $-20^{\circ} \mathrm{C}, 12$ months |
| Reagent 6 | $0.3 \mathrm{nmol} / \mu \mathrm{L}$ Standard | $2 \mathrm{~mL} \times 1$ vial | $5 \mathrm{~mL} \times 2$ vials | $-20^{\circ} \mathrm{C}, 12$ months |
| Reagent 7 | KRPH Buffer Solution | $55 \mathrm{~mL} \times 1$ vial | $60 \mathrm{~mL} \times 5$ vials | $-20^{\circ} \mathrm{C}, 12$ 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 $2-8^{\circ} \mathrm{C}$ 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 $0.3 \mathrm{nmol} / \mu \mathrm{L}$ standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: $0,0.06,0.12$, $0.15,0.18,0.21,0.24,0.3 \mathrm{nmol} / \mu \mathrm{L}$. Reference is as follows:

| Item | $(1)$ | $(2)$ | $(3)$ | $(4)$ | $(5)$ | $(6)$ | $(7)$ | $(8)$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Concentration (nmol $/ \boldsymbol{\mu} \mathbf{L})$ | $\mathbf{0}$ | $\mathbf{0 . 0 6}$ | $\mathbf{0 . 1 2}$ | $\mathbf{0 . 1 5}$ | $\mathbf{0 . 1 8}$ | $\mathbf{0 . 2 1}$ | $\mathbf{0 . 2 4}$ | $\mathbf{0 . 3}$ |
| $\mathbf{0 . 3} \mathbf{n m o l} / \boldsymbol{\mu}$ L standard $(\boldsymbol{\mu} \mathrm{L})$ | 0 | 40 | 80 | 100 | 120 | 140 | 160 | 200 |
| Double distilled water $(\boldsymbol{\mu} \mathrm{L})$ | 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 $200 \mu \mathrm{~L}$ of KRPH solution (including $2 \% \mathrm{BSA}$ ), add $100 \mu \mathrm{~L}$ of KRPH solution to the control well and sample well (including $2 \% \mathrm{BSA}$ ), then add $10 \mu \mathrm{~L}$ of $10 \mathrm{mmol} / \mathrm{L} 2-\mathrm{DG}$ to the sample well in the cell culture plate, and add $10 \mu \mathrm{~L}$ of KRPH solution to the control well. Incubate at $37^{\circ} \mathrm{C}$ for 30 min
(2) Wash cells for 3 times with $100 \mu \mathrm{~L}$ of KRPH solution, add $50 \mu \mathrm{~L}$ of acid reagent and stand at room temperature for 10 min , then add $50 \mu \mathrm{~L}$ alkali reagent.
(3) Standard well: Take $30 \mu \mathrm{~L}$ of standards with different concentrations into the corresponding fluorescence standard wells.

Sample well: Take $30 \mu \mathrm{~L}$ from sample well into the corresponding fluorescence wells.
Control well: Take $30 \mu \mathrm{~L}$ from control well of into the corresponding fluorescence wells.
(4) Add $170 \mu \mathrm{~L}$ of enzyme reagent working solution into each well.
(5) Incubate at $37^{\circ} \mathrm{C}$ 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 .

## 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 ( $\mathbf{y}=\mathbf{a x}+\mathbf{b}$ ) with graph software (or EXCEL).

The sample:
Glucose uptake content $(\mathrm{nmol} / \mu \mathrm{L})=\left(\mathrm{F}_{2}-\mathrm{F}_{1}-\mathrm{b}\right) \div \mathrm{a}$

## [Note]

$\mathrm{F}_{1}$ : The fluorescence intensity of control well.
$\mathrm{F}_{2}$ : 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 <br> $(\mathbf{n m o l} / \boldsymbol{\mu})$ | $\mathbf{0}$ | $\mathbf{0 . 0 6}$ | $\mathbf{0 . 1 2}$ | $\mathbf{0 . 1 5}$ | $\mathbf{0 . 1 8}$ | $\mathbf{0 . 2 1}$ | $\mathbf{0 . 2 4}$ | $\mathbf{0 . 3}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Fluorescence value | 5176 | 8677 | 11778 | 13332 | 14730 | 16049 | 17242 | 19704 |
|  | 5211 | 8643 | 11635 | 13276 | 14717 | 15968 | 17037 | 19603 |
| Average <br> fluorescence value | 5194 | 8660 | 11707 | 13304 | 14724 | 16009 | 17140 | 19654 |
| Absoluted <br> fluorescence value | 0 | 3467 | 6513 | 8111 | 9530 | 10815 | 11946 | 14460 |



## Appendix I Example Analysis

## Example analysis:

For 293 T cells $\left(0.7 \times 10^{\wedge} 6\right.$ cells $)$, carry the assay according to the operation steps. The results are as follows:
standard curve: $\mathrm{y}=48152 \mathrm{x}+521.17$, , the fluorescence value of the sample $\left(\mathrm{F}_{2}\right)$ is 9614 , the fluorescence value of the control (F1) is 4603 , and the calculation result is:

Glucose uptake content $(\mathrm{nmol} / \mu \mathrm{L})=(9614-4603-521.17) \div 48152=0.093 \mathrm{nmol} / \mu \mathrm{L}$
Detect 293 T 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.
