Glucose UptakeMicroplate Assay Kit

User Manual

Catalog # CAK1273

(Version 1.1A)

Determination of glucose uptake in whole cells and evaluation of effects of ligands or drugs on glucose transport.

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

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I. INTRODUCTION

Glucose uptake is an important biological tool for studying cell signaling and glucose metabolism.Glucose uptake has a variety of methods and transporters, and depends upon the metabolic demand of the cell type and availability of glucose. There are over ten different facilitated diffusion glucose transporters which transports glucose down its concentration gradient without ATP hydrolysis. In the kidneys, secondary active transport is used to uptake Glucose against its concentration gradient to ensure that very little glucose is excreted in urine.

Glucose Uptake Microplate Assay Kit provides a simple and direct procedure for measuring glucose uptake in whole cells and evaluation of effects of ligands or drugs on glucose transport. In this reaction,as with glucose, 2-DG can be taken up by glucose transporters and metabolized to 2-DG-6-phosphate (2-DG6P). 2-DG6P, however, cannot be further metabolized, and thus accumulates in the cells. The accumulated 2-DG6P is directly proportional to 2-DG (or glucose) uptake by cells.

II.KIT COMPONENTS

Component	Volume	Storage
Assay Buffer	Powder x 1	4 °C
Substrate	Powder x 1	4 °C
Lysis Buffer	10 ml x 1	4 °C
Reaction Buffer	5 ml x 1	4 °C
Enzyme	Powder x 1	4 °C
Dye Reagent A	Powder x 1	4 °C
Dye ReagentB	1 mlx 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
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Note:

Assay Buffer: add 10 mldistilled water to dissolve before use; store at -20 °C after reconstituting.

Enzyme: add 1 mlReaction Buffer to dissolve before use; store at -80 °C after reconstituting.

Substrate: add 1 mlReaction Buffer to dissolve before use; store at -20 °C after reconstituting.

Dye Reagent A: add 9 mldistilled water to dissolve before use; store at 4 °C after reconstituting.

Standard: add 1 mldistilled water to dissolve before use, the concentration will be 3mmol/L; store at -20 °C after reconstituting.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Centrifuge
6. Timer
7. Ice
8. 96-well culture plate

IV. SAMPLE PREPARATION

1. Seed 100 µl of 1-10 × 10^3 adherent cells (or 1-5 × 10^4 suspension cells)into each well of a 96-well culture plate (Not provide). Incubate for 4 hours or overnightat 37°C in a cell culture incubator.(Note: The cell number to be used depends on cell size and metabolicdemand of glucose.)
2. Incubate the cells with serum-less media for 4 hours or overnight toincrease their glucose demand.
3. Starve the cells by preincubating with 100 µlAssay Buffer for 40 minutes.Add any drugs or experimental treatments to the starvation media at thisstep if desired. Make sure to include a control group without anyexperimental conditions.
4. Add 10 µlSubstrate to each well. Incubate for 20 minutes ordesired time.
5. Remove the media. Then wash the cells 3 times with 150 µl of coldPBS to remove excess Substrate. Each wash should be performed for 30seconds without shaking, try not to disturb the cells.
6. Transfer 100 µl of Lysis Buffer to each well with cells, place the plate on arotary shaker for 5 minutes, and then incubate the plate at 80°C for 10minutes.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	20 µl	20 µl	20 µl
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled water	--	--	20 µl
Enzyme	10 µl	10 µl	10 µl
Mix, cover the plate adhesive strip, incubate at 37°C for 30 minutes.			
Dye Reagent A	90 µl	90 µl	90 µl
Dye ReagentB	10 µl	10 µl	10 µl
Mix, measured at 450 nmimmediately and recordthe absorbance.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1.According to the quantity of cells

2-DG6P (µmol/10^4)=($C_{Standard}$×$V_{Standard}$)×(OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) / (N ×V_{Sample}/ V_{Lysis})

= 0.3×(OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) / N

$C_{Standard}$: the standard concentration, 3mmol/L = 3µmol/ml;

$V_{Standard}$: the volume of standard,20 µl = 0.02 ml;

V_{Sample}: the volume of sample, 20 µl = 0.02 ml;

W: the weight of sample, g;

N: the quantity of cells, N ×10^4;

V_{Lysis}: the volume of LysisBuffer, 0.1 ml.

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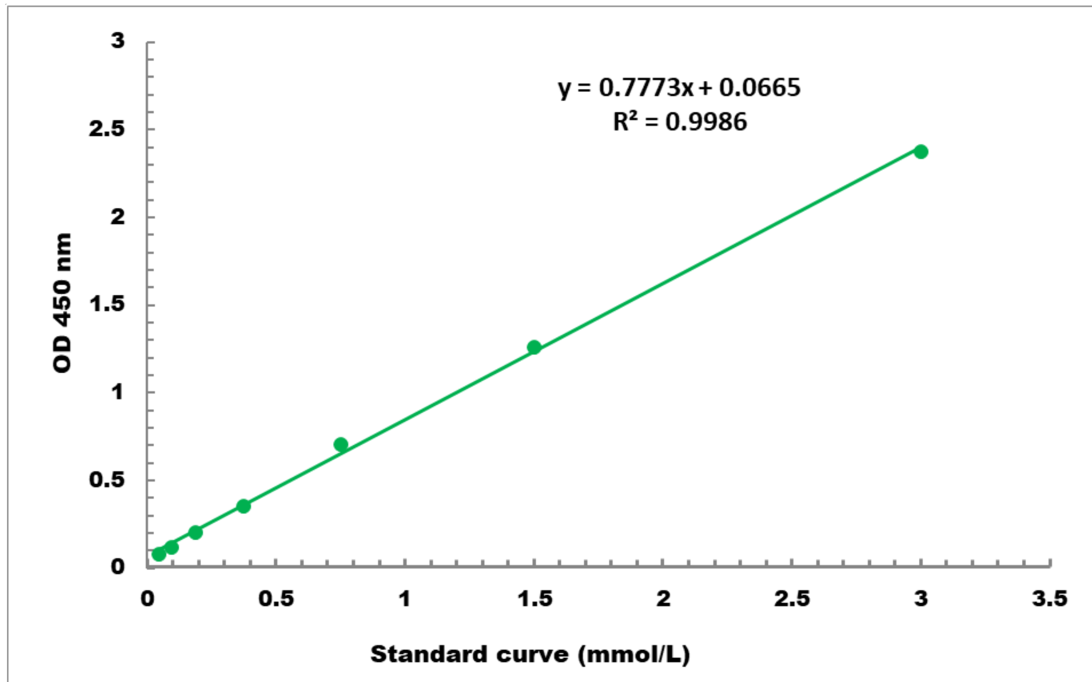
VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.03 mmol/L - 3 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES