ab136956

Glucose Uptake Assay Kit (Fluorometric)

Instructions for Use

For the sensitive and accurate measurement of Glucose uptake in various samples

This product is for research use only and is not intended for diagnostic use.

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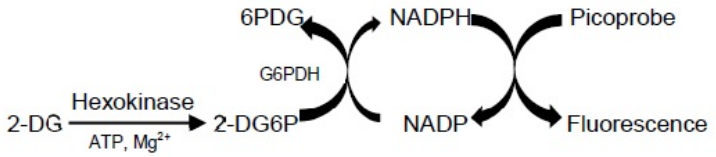
1. Overview

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-DG6P). 2-DG6P, however, cannot be further metabolized, and thus accumulates in the cells. The accumulated 2-DG6P is, therefore, directly proportional to 2-DG (or glucose) uptake by cells.

In Abcam's Glucose Uptake Assay Kit (Fluorometric), the accumulated 2-DG6P is enzymatically oxidized and coupled to a PicoProbe, which generates fluorescence in the presence of NADPH. This easy to use non-radioactive kit can detect glucose uptake as low as 50 pmol/well and can be used for a variety of cell types.

If you are looking for a more sensitive kit, we recommend using Glucose Uptake Colorimetric Assay Kit (ab136955), which contains an amplification step through NADPH recycling reaction.

Figure 1: Assay Procedure.



2. Protocol Summary

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⇩

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⇩

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⇩

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⇩

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3. Kits Components

Item	Quantity
Extraction Buffer	17 mL
Neutralization Buffer	1 mL
2-Deoxyglucose (2-DG, 10 mM)	1 mL
2-DG Uptake Assay Buffer	10 mL
Enzyme Mix (Lyophilized)	1 vial
PicoProbe (in DMSO)	0.2 mL
2-DG6P Standard (Lyophilized)	1 vial

4. Storage and Stability

Upon arrival, store the kit at-20°C and protected from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Warm all buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

5. Materials Required, Not Supplied

- 96-well plate with flat clear bottom, black wall plates are preferred for fluorescence reading
- Fluorescence plate reader
- Multi-channel pipette
- Krebs-Ringer-Phosphate-Hepes (KRPH) buffer: 20 mM Hepes, 5 mM KH_2PO_4, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 136 mM NaCl, 4.7 mM KCl, pH 7.4.
- BSA
- Distilled water or MilliQ

6. Assay Protocol

A. Reagent Preparation

1. Enzyme Mix:

Reconstitute with 220 µl Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles.

2. 2-DG6P Standard:

Reconstitute with 100 µl dH_2O to generate a 10 mM (10 nmol/µl) 2-DG6P Standard solution. Keep on ice while in use. Aliquot and store at –20°C. Use within two months.

3. PicoProbe:

Briefly warm at 37°C for 1 – 2 min prior use to completely melt the DMSO solution. Mix well. Store at –20°C after use.

B. Glucose Uptake Assay Protocol

1. 2-DG6P Standard Curve:

a) Dilute 2-DG6P 10 mM (10 nmol/µl) Standard to 0.1 mM (100 pmol/µl) by adding 5 µl of 10 mM 2-DG6P to 495 µl dH_2O and mix well (total volume 0.1 mM standard = 500 µl).

b) In a 96-well plate with round bottoms or in microcentrifuge tubes, prepare the following standard dilutions (total volume = 150 µl):

LABEL	END CONCENTRATION 2-DG6P IN WELL	2-DG6P [0. 1mM] amount (µl)	Assay buffer (µl)
A	0 pmol/well = 0µM	0	150
B	200 pmol/well = 4 µM	6	144
C	400 pmol/well = 8 µM	12	138
D	600 pmol/well = 12 µM	18	132
E	800 pmol/well = 16 µM	24	126
F	1000 pmol/well = 20 µM	30	120

c) Transfer in duplicate 50 µl of each the standard dilutions into the 96-well plate (flat bottoms) ready for the assay.

Note: make fresh dilution with the 10mM 2-DG6P standard stock solution each time.

2. Control and Sample Preparation:

This protocol has been optimized for **3T3-L1 adipocytes**. For other cell types, optimal incubation times and treatment protocols may vary from these conditions.

CONTROL PREPARATION – parallel sample well not treated with insulin or 2-Deoxyglucose.

a) Seed cells at a density of ~1500 cells/well in 100 µl culture medium in a 96-well plate and differentiate to mature adipocytes, maintain for another 4 days prior to use.

b) Wash adipocytes twice with PBS and starve in 100 µl serum free adipocyte medium overnight to increase glucose uptake.

c) Next day, wash cells 3X with PBS.

d) Starve the cells for glucose by pre-incubating them with 100 µl KRPH buffer containing 2 % BSA for 40 min.

e) Wash cells 3X with PBS.

f) Proceed to NAD(P) degradation step

SAMPLE (TREATED CELLS) PREPARATION – cells treated with desired method (for example, insulin)

g) Seed cells at a density of ~1500 cells/well in 100 µl culture medium in a 96-wp and differentiate to mature adipocytes, maintain for another 4 days prior to use.

h) Wash adipocytes twice with PBS and starve in 100 µl serum free adipocyte medium overnight to increase glucose uptake.

i) Next day, wash cells 3X with PBS.

j) Starve the cells for glucose by pre-incubating them with 100 µl KRPH buffer containing 2 % BSA for 40 min.

k) Stimulate cells with no insulin or with 1µM for 20 min to activate glucose transporter.

l) Add 10 µl of 10 mM 2-DG to the wells, mix by pipetting and incubate for 20 min.

m) Wash cells 3X PBS to remove exogenous 2-DG.

n) Proceed to NAD(P) degradation step.

3. Endogenous NAD(P) Degradation:

a) To degrade endogenous NAD(P) and to denature enzymes present in the sample, lyse cells with 90 µl of Extraction Buffer.

b) Freeze/thaw the cells once.

c) Heat samples at 85°C for 40 min.

If you have covered your plate with film or lid, you might find some condensation has formed. Simply spin the plate very briefly (~ 500 rpm 1 min) to get rid of the condensation bubbles before you take the lid or the cover off.

d) Cool the cell lysates on ice for 5 min and neutralize by adding 10 µl of Neutralization Buffer. Briefly spin the samples to ensure proper mixing of the reagents at ~ 500 rpm 1 – 2 minutes (or using soft spin cycle if available) and transfer supernatant to new tubes.

e) Add samples to wells in a 96 black well plate with clear bottoms. We recommend performing several different sample dilutions with the 2-DG Uptake Assay Buffer to ensure the readings fall within the standard curve. You can use 1 – 50 µl sample/well, adjusting to a 50 µl final volume with 2-DG Uptake Assay Buffer.

4. Reaction and Detection:

a) Reaction mix: Prepare Reaction Mix for each reaction.

	Reaction Mix
Assay Buffer	47 µl
PicoProbe**	2 µl
Enzyme Mix	2 µl

Mix enough reagents for the number of assays (control, samples and standards) to be performed. For example, to measure standards (6 dilutions x 2), control (1 sample x 2) and samples (1 sample x 2) – a total of 16 wells, plus one extra –, you will need:

	Reaction Mix A
Assay Buffer	47 µl x 17 = 799 µl
PicoProbe**	1 µl x 17 = 17 µl
Enzyme Mix	2 µl x 17 = 34 µl

Add 50 µl Reaction Mix into each well, mix by pipetting up and down.

*NOTE: for samples that have 2-DG uptake less than **100 pmol**, repeat the assay by reducing the probe volume to 0.5 µl per well to reduce reagent background and accordingly increase the volume of Assay Buffer.*

b) Plate reading: incubate plate at 37°C for 40 minutes, protected from light. Measure fluorescence at Ex/Em = 535/587 nm.

7. Data Analysis

Calculations:

a) Subtract the zero pmol standard from all standard readings.

b) Plot the 2-DG6P Standard Curve.

c) Correct sample background by subtracting the value derived from the untreated cells (Control = cells not treated with insulin and 2-DG) from all sample readings

Note: The background reading can be significant and must be subtracted from all sample readings.

d) 2-DG concentration of the test samples, which is proportional to accumulated 2-DG6P can then, be calculated using the following formula:

2-DG uptake = Sa/Sv (pmol/µl or nmol/ml or µM)

Where:

Sa is the amount of 2-DG6P (in pmol) in the sample well calculated from Standard Curve.

Sv is sample volume (in µl) added into the sample wells.

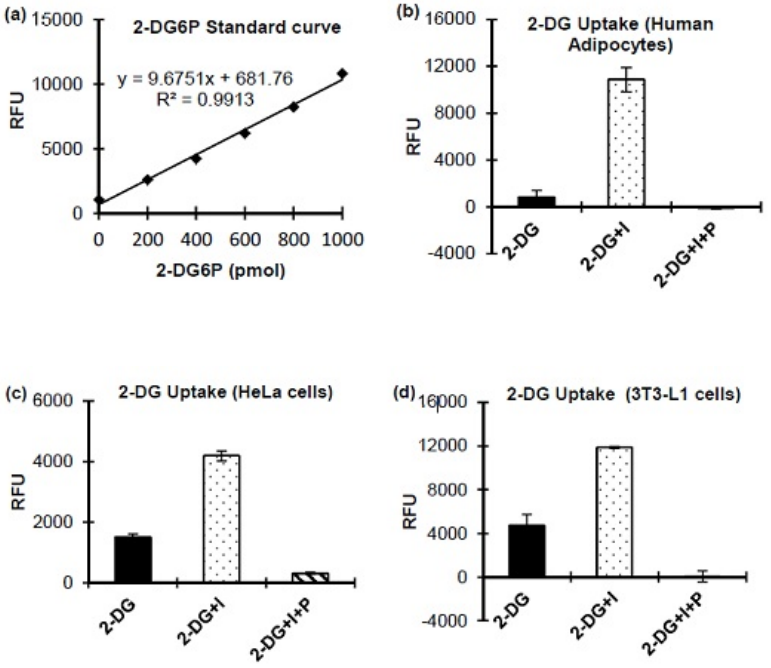

Figure 2. (a) 2-DG6P Standard curve **(b), (c)** and **(d)** 2-DG uptake in human adipocytes, Hela Cells and 3T3-L1 cells respectively. **I=**Insulin; **P=**Phloretin (glucose transport inhibitor). The concentration and duration of Phloretin treatment depends on the cell type and the level of expression of GLUT transporter. For guidance, we have used 200 µM for 20-30 minutes, but the time and concentration should be optimised for your own assay.

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

For all technical or commercial enquiries please go to:
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