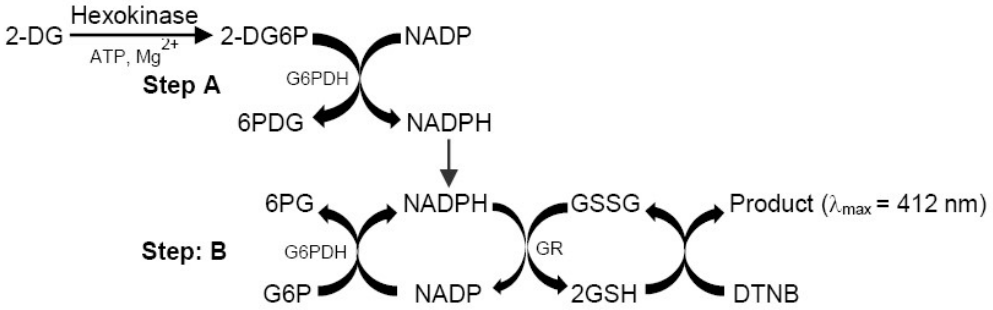
Glucose Uptake Assay Kit I

Colorimetric, highly sensitive assay to detect glucose uptake as low as 10 pmol/well in adherent or suspension cells

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K676
Description	Glucose uptake is an important biological tool for studying cell signaling and glucose metabolism. Of many different methods available for measuring glucose uptake, 2-deoxyglucose (2-DG) has been most 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 directly proportional to 2-DG (or glucose) uptake by cells. In PromoKine's Glucose Uptake Assay Kit I, 2-DG6P is oxidized to generate NADPH, which is determined by an enzymatic recycling amplification reaction. This easy to use non-radioactive kit is highly sensitive and can detect glucose uptake as low as 10 pmol/sample.  **Assay Procedure**: Step A: 2-DG oxidation to generate NADPH; Step B: NADPH recycling amplification reaction
Quantity	100 assays
Applications	• Measurement of glucose uptake in response to insulin, growth factors, cytokines, mitogens and nutrients, etc. • Analysis of glucose metabolism and cell signaling in various cell types • Screening anti-diabetic drugs
Sample Type	• Cell culture: Adherent or suspension cells

Components	**Quantity**	**Color Code**
Extraction Buffer	17 ml	NM
Neutralization Buffer	2.5 ml	Clear
2-Deoxyglucose (2-DG, 10 mM)	1 ml	Purple
2-DG Uptake Assay Buffer	25 ml	WM
2-DG6P Standard (lyophilized)	1 vial	Yellow
Glutathione Reductase	2 x 25 µl	Green
Enzyme Mix (lyophilized)	1 vial	Orange
Recycling Mix (lyophilized)	1 vial	Blue
Substrate-DTNB (lyophilized)	2 vials	Red

User Supplied Reagents & Equipment	• 96-well plate with flat clear bottom • Multi-well spectrophotometer (ELISA reader) • Multi-channel pipette • Plate sealing tape • 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
Storage and Reagents Preparation	Store kit at –20°C, protected from light. Warm all Buffers to room temperature before use. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **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.
- **Recycling 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-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. Store at –20°C. Use within two months.
- **Gluthathione Reductase**: Add 1.1 ml Assay Buffer. Mix well, aliquot and store at –20°C. Avoid repeated freeze/thaw cycles.
- **Substrate-DTNB**: Reconstitute with 1 ml Assay Buffer. Dissolve completely by pipetting up and down. Store at –20°C. Reconstituted substrate is stable for 2 months.

Assay Protocol

1. 2-DG6P Standard Curve: Dilute 2-DG6P Standard to 0.1 mM (100 pmol/µl) by adding 10 µl of 10 mM 2-DG6P to 990 µl Assay Buffer and mix well. Dilute further to 0.01 mM (10 pmol/µl) by adding 50 µl of 0.1 mM 2-DG6P to 450 µl Assay Buffer. Add 0, 2, 4, 6, 8 and 10 µl into a series of well on a 96 well plate in duplicate to generate 0, 20, 40, 60, 80 and 100 pmol/well of 2-DG6P standard. Adjust volume to 50 µl/well with Assay Buffer. Note: make fresh dilution with the 10 mM 2-DG6P standard stock solution each time.

2. Sample Preparation: Treat cells with desired method. Example: For 3T3-L1 adipocytes, cells were seeded at a density of ~1500 cells per well in a 96-well plate, differentiated, then maintained for another 4 days prior to use. To assay glucose uptake, adipocytes were washed twice with PBS and starved in 100 µl serum-free adipocyte medium overnight (to increase glucose uptake), then rewashed 3X with PBS. The cells were starved for glucose by preincubating with 100 µl Krebs-Ringer-Phosphate-HEPES (KRPH) buffer containing 2% BSA for 40 minutes then stimulated or not (control, see below) with 1 µM insulin for 20 minutes to activate the glucose transporter. 10 µl of 10 mM 2-DG was added and the cells incubated for 20 minutes.

Wash cells 3X with PBS to remove exogenous 2-DG. To degrade endogenous NAD(P) and to denature enzymes, lyse cells with 80 µl of Extraction Buffer, freeze/thaw once and heat at 85°C for 40 minutes. Cool the cell lysate on ice for 5 minutes and neutralize by adding 10 µl of Neutralization Buffer. Briefly spin & dilute 1:10 by adding 45 µl of Assay Buffer to 5 µl sample. Add 1-50 µl sample per well. Adjust final volume to 50 µl with Assay Buffer. Notes:

1) As a control, prepare a parallel sample well not treated with insulin and 2-DG.

2) For other cell types, optimal incubation times and treatment protocols may vary from these conditions. We suggest testing several doses of cell lysate to ensure the readings are within the standard curve range. We suggest starving cells overnight in serum-free medium to increase 2-DG uptake.

3. Reaction Mix:

a. NADPH Generation: Mix enough reagents for the number of assays (Control, samples and standards) to be performed. For each well, prepare 10 µl Reaction Mix A:

	Reaction Mix A
Assay Buffer	8 µl
Enzyme Mix	2 µl

Add 10 µl Reaction Mix A into each well. Mix and incubate at 37°C for 1 hour.

b. NADP Degradation: To degrade unused NADP (Figure 1, step A), add 90 µl of extraction buffer to each well, seal with aluminum sealing tape and heat at 90°C for 40 minutes. Cool on ice for 5 minutes and add 12 µl of neutralization buffer.

c. Recycling Amplification Reaction: For each well (control, standards and samples), prepare 38 µl recycling reaction mix B:

	Reaction Mix B
Glutathione Reductase	20 µl
Substrate (DTNB)	16 µl
Recycling Mix	2 µl

Mix. Add 38 µl Reaction Mix B into each well; Mix well.

4. Measurement: Measure absorbance at 412 nm in microplate reader at 37°C every 5 minutes until the 100 pmol standard reaches 1.5-2.0 OD. Take an endpoint reading of all samples and standards.

5. Calculation: Subtract the 0 pmol standard from all standard readings. Plot the 2-DG6P Standard Curve. Correct sample background by subtracting the value derived from the untreated cells (Control i.e. 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). 2-DG concentration of the test samples, which is proportional to accumulated 2-DG6P, can then be calculated. **2-DG uptake = Sa/Sv (pmol/µl or nmol/ml or µM)** Where: Sa is the amount of 2-DG6P (in pmol) in sample well calculated from Standard Curve. Sv is sample volume (in µl) added into the sample well.
Intended Use	For in vitro research use only. Not for diagnostic or therapeutic procedures.

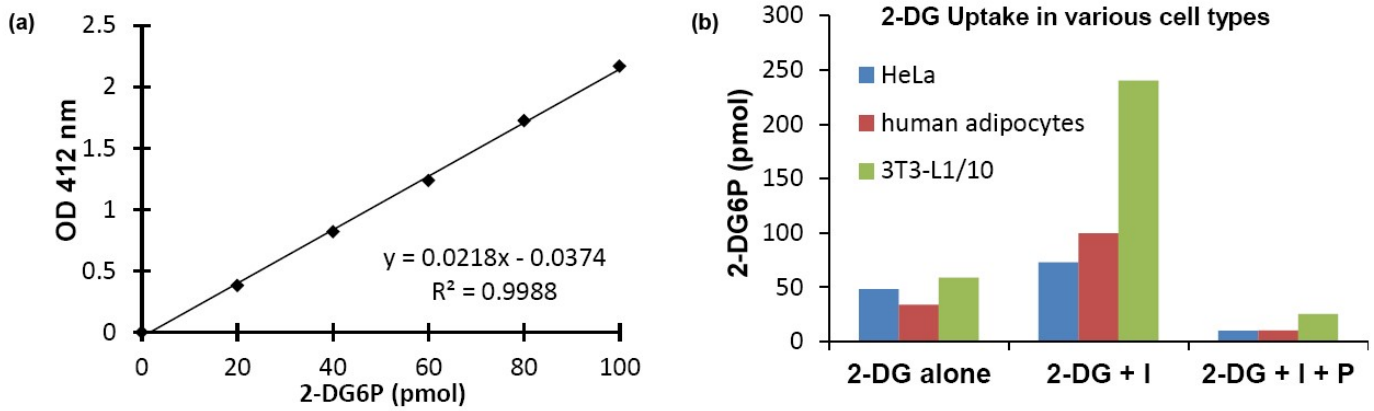


Figure: (a) 2-DG6P Standard curve (b) 2-DG uptake in 3T3-L1, human adipocyte and Hela cells. To scale on the same graph, data from 3T3-L1 cells is plotted at 10% of true value. Assays were performed following kit protocol. 2-DG = 2-deoxyglucose, I = Insulin; P = Phloretin.

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