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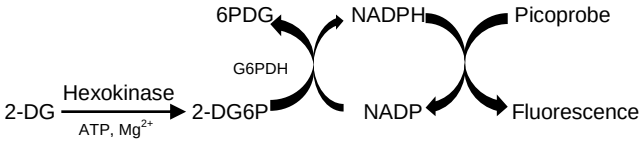
Glucose Uptake Fluorometric Assay Kit

rev. 8/18

(Catalog # K666-100; 100 assays; Store at -20°C)

I. Introduction:

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 BioVision's glucose uptake fluorometric assay kit, the accumulated 2-DG6P is enzymatically oxidized and coupled to BioVision's 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.



II. Application:

- 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.

III. Sample Type:

- Adherent or suspension cells cultured in 96-well microtiter plate.

IV. Kit Contents:

Components	K666-100	Cap Code	Part Number
Extraction Buffer	17 ml	NM	K666-100-1
Neutralization Buffer	1 ml	Clear	K666-100-2
2-Deoxyglucose (2-DG, 10 mM)	1 ml	Purple	K666-100-3
2-DG Uptake Assay Buffer	10 ml	WM	K666-100-4
Enzyme Mix (Lyophilized)	1 Vial	Green	K666-100-5
PicoProbe™ (in DMSO)	0.2 ml	Blue	K666-100-6
2-DG6P Standard (Lyophilized)	1 Vial	Yellow	K666-100-7

V. User Supplied Reagents and Equipment:

- Two 96-well plates: A. sterile, cell culture treated and B. black, flat bottom, non-sterile microplate
- Fluorescence plate reader
- Multi-channel pipette
- 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.

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm all Buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

- **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-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.
- **PicoProbe™:** Briefly warm at 37°C for 1-2 min to completely melt the DMSO solution. Mix well, store at –20°C after use.

VIII. Glucose Uptake Assay Protocol:

1. 2-DG6P Standard Curve: Dilute 2-DG6P Standard to 0.1 mM by adding 10 µl of 2-DG6P to 990 µl dH2O and mix well. Add 0, 2, 4, 6, 8 and 10 µl into a series of well on a 96 well plate in duplicate to generate 0, 200, 400, 600, 800 and 1000 pmol/well of 2-DG6P standard. Adjust volume to 50 µl/well with Assay Buffer.

2. Sample Preparation:

Treat cells with desired method. For 3T3-L1 adipocytes, for example, seed cells at a density of ~1500 cells per well in a 96 well plate and differentiate to mature adipocytes, maintain for another 4 days prior to use. To assay glucose uptake, wash adipocytes twice with PBS and starve overnight in 100 µl serum free adipocyte medium (to increase glucose uptake). Wash cells 3X with PBS. Starve the cells for glucose by preincubating with 100 µl Krebs-Ringer-Phosphate-Hepes (KRPH) buffer containing 2 % BSA for 40 min. Stimulate with or without insulin (1 µM) for 20 min to activate glucose transporter. Add 10 µl of 10 mM 2-DG, mix and incubate for 20 min. **Note:** For control reading for each sample, do not treat cells with insulin and 2-DG. Wash cells 3X with PBS to remove exogenous 2-DG. To degrade endogenous NAD(P) and to denature enzymes, lyse cells with 90 µl of Extraction Buffer, freeze/thaw once and heat at 85°C for 40 min. Cool the cell lysate on ice for 5 min and neutralize by adding 10 µl of Neutralization Buffer. Add up to 50 µl sample per well, adjust final volume to 50 µl with Assay Buffer.

Notes: 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:** Mix enough reagents for the number of assays (control, samples and standards) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix
Assay Buffer	47 µl
PicoProbe**	2 µl
Enzyme Mix	1 µl

** 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.

4. **Measurement:** Mix well. Incubate at 37°C for 40 min., protected from light. Measure fluorescence at Ex/Em = 535/587 nm.

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 the sample well calculated from Standard Curve.
Sv is sample volume (in µl) added into the sample wells.

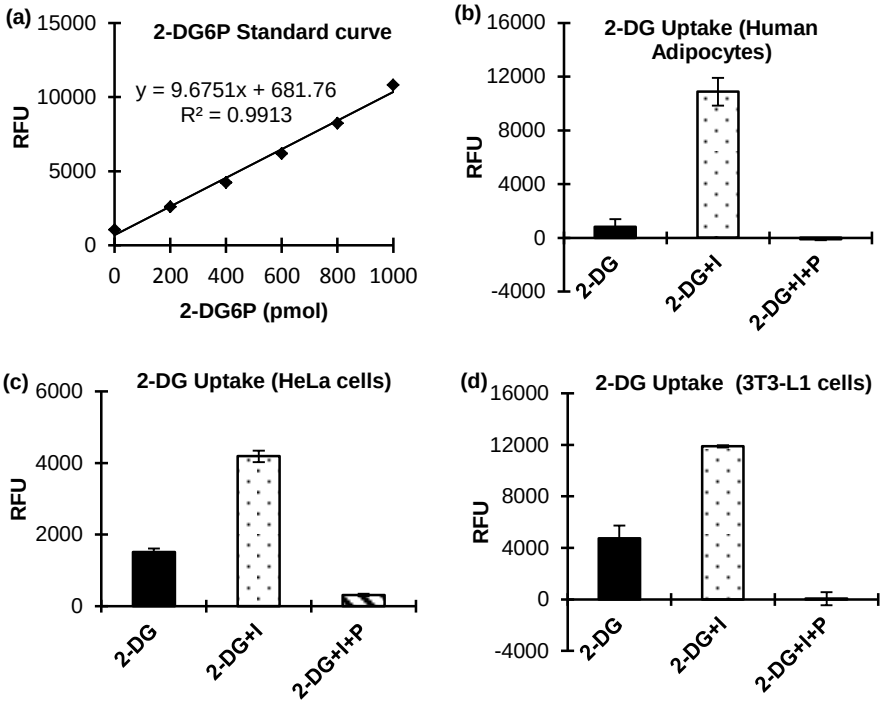


Figure: **(a)** 2-DG6P Standard curve **(b), (c)** and **(d)** 2-DG uptake in human adipocytes, Hela Cells and 3T3-L1 cells respectively. Assays were performed following kit protocol. **I**=Insulin; **P**=Phloretin.

IX. RELATED PRODUCTS:

Glucose and Sucrose Assay Kit
Glucose Assay kit
NAD/NADH Quantification Kit
NADP/NADPH Quantification Kit
Glucose Dehydrogenase Activity Assay Kit
Glucose-6-Phosphate Dehydrogenase Assay Kit
Maltose and Glucose Assay Kit
PicoProbe™ Glucose-6-Phosphate Assay Kit
Adipogenesis Assay Kit

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