NON-RADIOACTIVE

2DG Glucose Uptake Assay

High Sensitive Kit & Broad Range Kit

Studies of the effects of insulin and other growth factors on cells behavior and metabolism often include measurements of glucose uptake following cell stimulation. Glucose uptake experiments are typically performed using radioactive non-metabolizable glucose analogs such as ^{3}H-2-deoxyglucose (2DG). However, the use of radioisotopes is not available to all labs and is subject to many restrictions.

High Sensitive Kit — 2-Deoxyglucose uptake measurement kit (Chromogenic)

Sensitive, accurate, and safe measurement of glucose uptake (2DG) by cultured cells is now available to all laboratories. No radiation permit required !

- **Picomole sensitivity**
- **Advanced recycling enzymatic amplification**
- **Photometric detection (420 nm)**
- **No wash, automation friendly assay protocol**
- **Optimized for 96-well culture plates**
- **Needs no correction for extracellular 2DG**

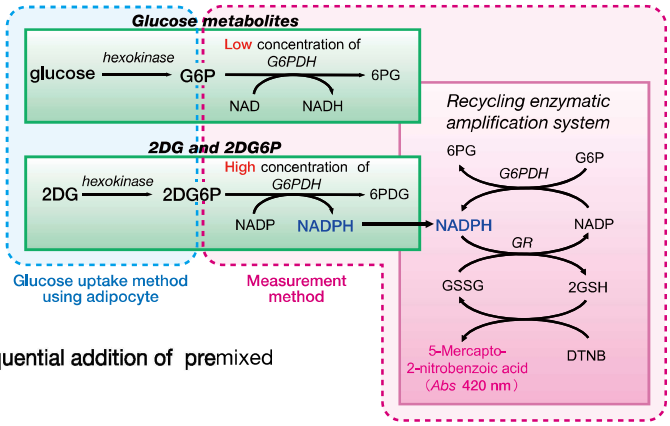
Assay Principle: A recycling enzymatic amplification system measures NADPH produced by the *in vitro* oxidation of 2DG6P accumulated in cells following 2DG uptake.

1) So as not to effect glucose metabolism, only a small amount of 2DG is added to live cells. Incorporated 2DG is converted by cell metabolism to 2D6GP, which accumulates in cells. Cell lysates are then prepared.

2) To eliminate detection of G6P, G6P is oxidized (to 6PG) with NAD^{+} and a low concentration of G6PDH.

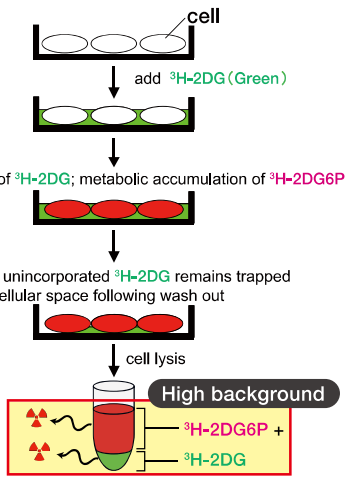
3) 2DG6P levels are quantitated by measuring the amount of NADPH produced during 2DG6P oxidation (with NADP^{+} and a high concentration of G6PDH) in a phtometric recycling amplification/detection system.

All reaction steps are conveniently performed in a single well by the sequential addition of premixed reagents. Ideal for assay automation.



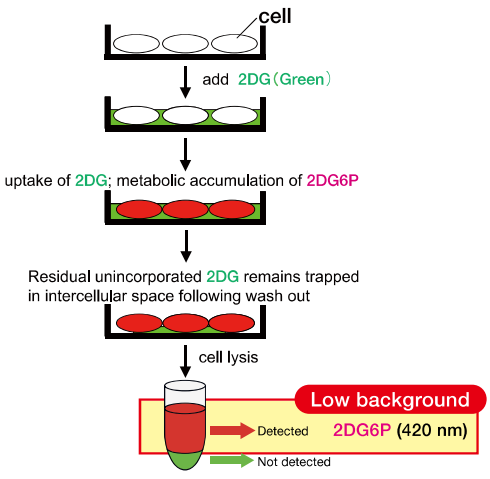
Enzymatic assay eliminates measurement errors due to unincorporated ^{3}H-2DG

RI method



High assay background caused by inability to distinguish between cellular ^{3}H-2DG6P and residual unincorporated ^{3}H-2DG.

2-Deoxyglucose uptake measurement kit



Low assay background because residual unincorporated 2DG is not converted to 2DG6P.

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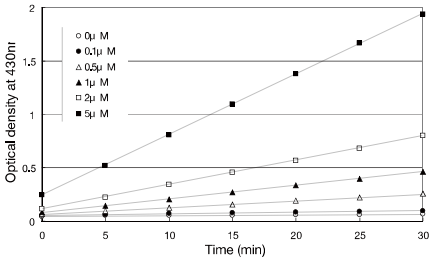
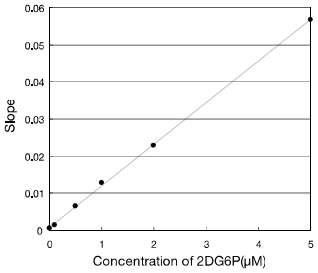
2DG Glucose Uptake Assay

High Sensitive Kit — 2-Deoxyglucose uptake measurement kit (Chromogenic)

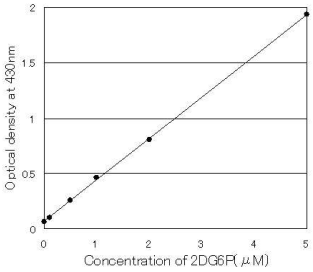
Assay performance using known concentrations of 2DG6P



Optical Density (OD) vs. time at various concentrations of 2DG6P

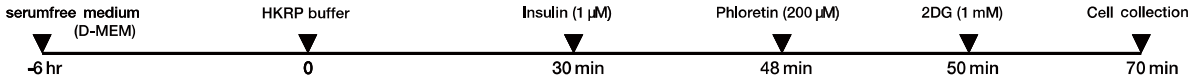


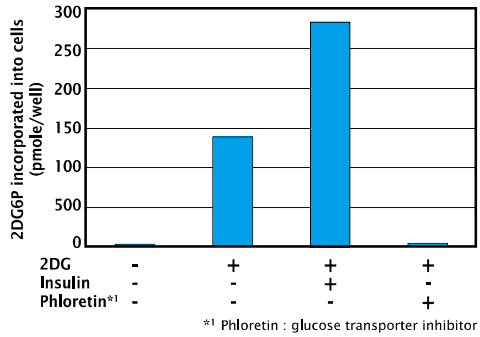
Assay calibration curve – Kinetic Mode (30 minutes incubation)



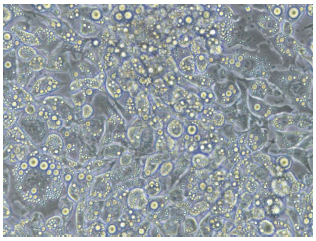
Assay calibration curve – Endpoint Mode (30 minutes incubation)

Experimental Example 1 – 2-deoxyglucose (2DG) uptake by 3T3-L1 cells



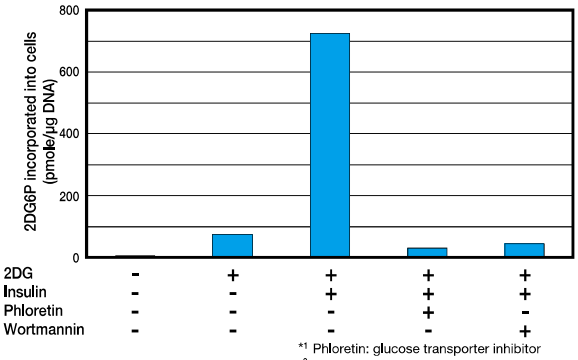


*1 Phloretin : glucose transporter inhibitor

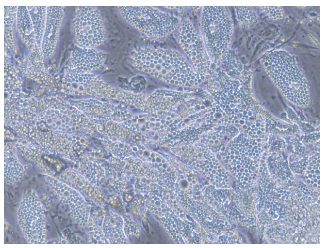


Mouse Preadipocyte 3T3-L1
Medium : D-MEM + 10%CS, D-MEM +10%FBS

Experimental Example 2 - 2-deoxyglucose (2DG) uptake by human adipocytes



*1 Phloretin: glucose transporter inhibitor
*2 Wortmannin: PI3 kinase inhibitor



Human Preadipocyte —subcutaneous fat tissue
(ScienCell Research Laboratories Cat.# 7220)
Medium : Visceral Adipocyte Culture Medium ver.2
(Cat.# PMC-VACM2-COS)

Broad Range Kit Glucose Cellular Uptake Measurement Kit (Fluorometric)

A complete reagent set to measure 2DG in cells after cell lysis by sonication

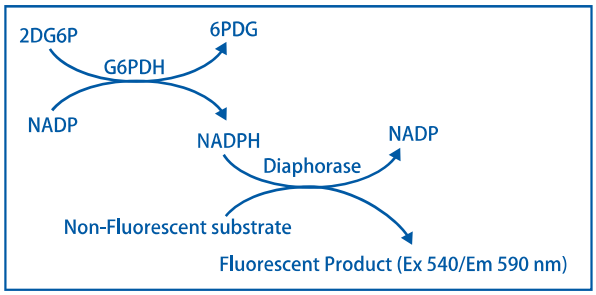


Figure 1: Scheme of 2DG Detection

- Assay measures amount of 2-deoxyglucose (2DG) (Glucose analog) uptake.
- Like glucose, 2DG taken up by cells is rapidly phosphorylated by hexokinase to 2-deoxyglucose-6-phosphate (2DG6P). However, 2DG6P is not further metabolized and accumulates in cells.
- Cell lysates are assayed for 2DG6P levels in a coupled enzymatic re-dox reaction that produces a fluorescent signal of intensity proportional to the amount of accumulated 2DG6P.
- 2DG levels in cell lysate samples are calculated by comparing their fluorescence intensity to a standard curve produced with known amounts of 2DG6P.

Experimental Results

2DG uptake by insulin-stimulated adipocytes following the differentiation of 3T3-L1 cells in culture.

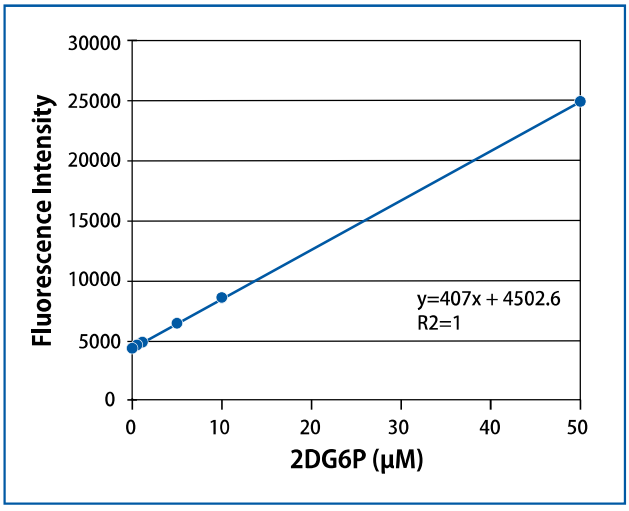


Figure 2: Calibration curve with 2DG6P Solution

Additives	Sample A	Sample B	Sample C
Insulin	−	−	+
2DG	−	+	+

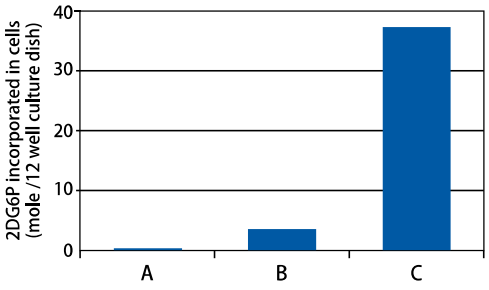
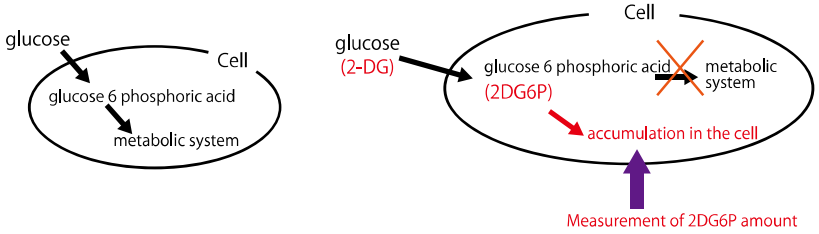


Figure 3: Measurement results

A small amount of 2DG is administered into animals or cultured cells, and endogenous glucose and glucose-6phosphate (G6P) in tissues or cells is oxidized in the presence of a low concentration of G6PDH. 2DG-6-phosphate (2DG6P) accumulated in cells is then oxidized in the presence of a high concentration of G6PDH. NADPH produced from 2DG6P and G6PDH is quantified at 420 nm with the use of a recycling amplification enzymatic-photometric system. The novel enzymatic method can quantify 2DG or 2DG6P in the range of 5-80 pmol. As all enzyme reactions are performed in one 96-well microplate by the sequential addition of reagents, this method can be adopted for industrial robots. This method is useful for the screening of anti-diabetic drugs as well as for research in glucose metabolism and insulin signaling.



Comparison of Cosmo Bio 2DG Measurement kits

	Glucose Cellular Uptake Measurement Kit, Broad Range, Fluorometric Cat. No. CSR-MBR-PMG-K01E	2-Deoxyglucose (2DG) Uptake Measurement Kit Cat. No. CSR-OKP-PMG-K01E
Assay Format	Non-radioactive	Non-radioactive
Detection Method	Fluorometric (Ex 540 nm/Em 590 nm)	Chromogenic (420 nm)
Assay time (after sample prep)	2 hours	5-7 hours
Measurement Range	Broad (0-50 µM 2DG6P)	High sensitivity (0 to 5 µM 2DG6)
Features	• Fast • Convenient • Single step suitable for high sample throughput	• Sensitivity comparable with radioactive assays • High accuracy • High precision

Kit Components

High Sensitivity Cat. No.: CSR-OKP-PMG-K01E		
Reagent	Volume	Quantity
Solution A	3,400 µL*	1 vial
Solution B (Acid solution)	1,000 µL	1 vial
Solution C (Acid neutralizing solution)	1,000 µL	1 vial
Solution D	1,600 µL*	1 vial
Solution E (Alkali solution)	1,000 µL	1 vial
Solution F (Alkali Neutralizing solution)	1,000 µL	1 vial
Solution G	2,000 µL	1 vial
1 mM 2DG6P solution	500 µL	1 vial
Sample diluent buffer Concentrate (100-fold concentrated solution)	3 mL	1 vial
Substrate buffer	11 mL	1 vial
DTNB Substrate (powder)		5 vials
Low G6PDH	25 µL	1 vial
High G6PDH	250 µL	1 vial
GR	20 µL	1 vial

* half for CSR-OKP-PMG-K01TE

Broad Cat. No.: CSR-MBR-PMG-K01E		
Reagent	Volume	Quantity
2DG6P Solution (1 mM)	500 µL	1 vial
Sample Diluent Buffer Concentrate (100x)	5 mL	1 vial
Substrate Buffer	9 mL	3 vial
Fluorescent Substrate	120 µL	1 vial
Enzyme Solution	270 µL	1 vial

Storage: -20℃

Ordering Information

Product Description	Cat. No.	Size
2-Deoxyglucose (2DG) Uptake Measurement Kit (Chromogenic)	CSR-OKP-PMG-K01	1 kit (50 tests)
Glucose Cellular Uptake Measurement Kit (Broad Range, Fluorometric)	CSR-MBR-PMG-K01	1 kit (100 tests)

Shipping Condition: Dry Ice

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