Ver.251101

Glucose Uptake Colorimetric (Colorimetric) Assay Kit

OGC267-01 (100Tests)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

The **Glucose Uptake Colorimetric Assay Kit** is designed to quantitatively measure glucose uptake in cultured cells based on a **colorimetric detection method**. The assay uses the glucose analog **2-deoxyglucose (2-DG)**, which is taken up by cells and phosphorylated to **2-DG-6-phosphate (2-DG6P)** but cannot undergo further glycolytic metabolism. The accumulated 2-DG6P is then specifically detected through an enzymatic reaction that produces a measurable **color change**, with absorbance typically read at 412 nm.

Kit components

Reagent	Volume	Storage
Assay Buffer III	25mL	-20°C
Extraction Buffer I	17mL	-20°C
Neutralization Buffer II	2.5mL	-20°C
Enzyme Mix III	1 Vial	-20°C
Glutathione Reductase	2 x 25µL	-20°C
Recycling Mix	1 Vial	-20°C
DTNB	2 x 1 Vial	-20°C
2-Deoxyglucose	1mL	-20°C
2-DG6P Standard	1 Vial	-20°C

Storage:

Store kit at -20°C in the dark immediately on receipt and check below storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted. Reconstituted components are stable for 2 months. Aliquot components in working volumes before storing at the recommended temperature. Avoid repeated freeze thaws of reagents.

Reagents and Equipment Required but Not Provided

- Microplate reader capable of measuring absorbance (OD) at 412 nm wavelength.
- Plate sealing tape.
- 96 well plate with clear flat bottom.
- KRPH (Krebs-Ringer-Phosphate-Hepes) buffer: 20mM HEPES,5Mm KH_2PO_4,1Mm $MgSO_4$, 1mM $CaCl_2$, 136 mM NaCl, 4.7 mM KCl, pH 7.4
- Adipocyte culture medium (serum free and serum supplemented) – if using a different cell type, use appropriate culture medium.
- Insulin (glucose transporter activator).

Precaution

- To avoid contamination, use clean laboratory wares. Use clean, dry disposable pipette tips for dispensing. Close reagent bottles immediately after use.
- Avoid direct exposure of reagent to light. Do not blow into the reagent bottles.

1. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

1.1 Assay Buffer III: Ready to use as supplied. Equilibrate to room temperature before use.

1.2 Extraction Buffer I: Ready to use as supplied. Equilibrate to room temperature before use.

1.3 Neutralization Buffer II: Ready to use as supplied. Equilibrate to room temperature before use.

1.4 Enzyme Mix III: Reconstitute with 220µL Assay Buffer III. Keep on ice during assay. Aliquot Enzyme Mix III so that you have enough volume to perform the desired number of assays.

1.5 Glutathione Reductase: Reconstitute with 1.1 mL Assay Buffer III. Aliquot enzyme so that you have enough volume to perform the desired number of assays.

1.6 Recycling Mix: Reconstitute with 220µL Assay Buffer III. Aliquot the mix so that you have enough volume to perform the desired number of assays.
1.7 DTNB: Reconstitute with 1 mL Assay Buffer III. Aliquot DTNB so that you have enough volume to perform the desired number of assays.
1.8 2-Deoxyglucose: Ready to use as supplied. Equilibrate to room temperature before use.
1.9 2-DG6P Standard: Reconstitute with 100µL distilled water to generate a 10 mM (10 nmol/µL) 2-DG6P solution. Aliquot standard so that you have enough volume to perform the desired number of assays.

2. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

2.1 Prepare 500µL of 0.1mM (100 pmol/µL) 2-DG6P Standard by adding 5µL of the 10mM 2-DG6P Standard in 495µL Assay Buffer III, mix well.
2.2 Prepare 500µL of 0.01mM (10 pmol/µL) 2-DG6P by diluting 50µL of 0.1mM 2-DG6P standard (step 2.1) in 450µL Assay Buffer III
2.3 Using 0.01mM 2-DG6P standard (step 2.2), prepare standard curve dilutions as described below, in a microplate or microcentrifuge tubes:

Standard	2-DG6P Standard (µL)	Assay Buffer III (µL)	Final volume standard in well (µL)	End amount 2-DG6P in well (pmol/well)
1	0	150	50	0
2	6	144	50	20
3	12	138	50	40
4	18	132	50	60
5	24	126	50	80
6	30	120	50	100

Operation Procedures

3. Sample Preparation

- This protocol has been optimized for 3T3-L1 adipocytes. For other cell types, optimal incubation times and treatment protocol may vary, and should be set by the researcher.
- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C after step 4.1.5. When ready to test, thaw samples on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected. Avoid multiple freeze-thaws.

3.1 Seed Cells:

3.1.1 Seed cells at a density of ~ 1,500 – 2,000 3T3-L1 cells/well in 100µL culture medium in a sterile 96-well plate and differentiate to mature adipocytes and maintain for another 4 days prior to use.
3.1.2 Wash adipocytes twice with PBS and starve in 100µL serum free adipocyte medium overnight to increase glucose uptake. The time (overnight) can be reduced for cells which will not tolerate overnight starvation.
3.1.3 Next day, wash cells 3X in PBS.
3.1.4 Starve cells for glucose by pre-incubating with 100µL KRPH buffer containing 2 % BSA for 40 minutes.
Note: Prepare enough cells to be able to set up control (untreated) and treated Cells.

3.2 Sample preparation:

3.2.1 Sample background control (untreated) cells: Wash glucose starved cells (step 3.1.4)3X with PBS. Proceed to Step 4.1. Do not add 2-DG.
3.2.2 Insulin stimulated cells: stimulate glucose starved cells in KRPH buffer containing 2%BSA (step 3.1.4) with 1µM insulin for 20 minutes, to activate glucose transporter. Total volume/well = 100µL.
3.2.3 Control (untreated); non-insulin stimulated cells.

3.2.4 Add 10µL of 10mM 2-DG to insulin stimulated cells and non-insulin stimulated control (untreated) cells.

3.2.5 Incubate for 20 minutes.

3.2.6 Wash cells 3X with PBS to remove exogenous 2-DG.

Note: The insulin incubation provides quick stimulation of glucose uptake via GLUT transporters.

4. Assay Procedure

4.1 Endogenous NAD(P) degradation:

4.1.1 Lyse all, samples background control, control (untreated) and insulin treated cells with 80µL Extraction Buffer I by pipetting up and down to release cells. If using a bigger culture surface, you can do this step with a cell scraper.

4.1.2 Freeze/thaw cell lysates once and heat at 85°C for 40 minutes.

Note: This step will degrade endogenous NAD(P) and denature enzymes in the samples to minimize background signal.

Note: At this point, samples can be transferred to microcentrifuge tubes for ease of handling or can be left in the microplate if preferred.

Note: In case of plate covered with film or lid, some condensation bubbles may form, spin the plate briefly (~ 500 rpm 1 minute) to get rid of the condensation bubbles before taking the lid or cover off.

4.1.3 Cool cell lysate on ice for 5 minutes.

4.1.4 Neutralize sample by adding 10µL of Neutralization Buffer II. Briefly spin the samples to ensure proper mixing of reagents (~ 500 rpm for 1 – 2 minutes, or using soft spin cycle).

4.1.5 Transfer supernatant to new tubes.

4.1.6 Samples may be diluted 1:10 times by adding 45µL Assay Buffer III in 5µL sample.

4.2 Reaction wells set up:

4.2.1 Standard wells = 50µL standard dilutions.

Sample background control (no insulin and no 2-DG treated) = 1 -50µL (adjust volume to 50µL/well with Assay Buffer III).

4.2.3 Insulin stimulated sample well = 1-50µL (adjust volume to 50µL/well with Assay Buffer

4.2.4 Control sample wells (no insulin, 2-DG treated) = 1-50µL (adjust volume to 50µL/well with Assay Buffer III.

4.3 Reaction mix:

4.3.1 Prepare 10µL/well of Reaction Mix A (NADPH generation) for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency.

Component	Reaction A Mix (µL)
Assay Buffer III	8
Enzyme Mix III	2

4.3.2 Add 10µL of Reaction Mix A into each well of standard, controls and sample wells.

4.3.3. Mix and incubate at 37°C for 1 hour.

4.3.4. Add 90µL Extraction Buffer I to each well, seal the microplate with an aluminium sealing tape and heat it to 90°C for 40 minutes to degrade any unused NADP left.

4.3.5. Cool plate on ice for 5 minutes and neutralize reaction by adding 12µL of Neutralization Buffer II.

4.3.6. Prepare 38µL of Reaction Mix B (recycling amplification reaction) for each reaction.

Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix B to ensure consistency.

Component	Reaction B Mix (µL)
Glutathione Reductase	20
DTNB	16
Recycling Mix	2

4.3.7 Add 38µL Reaction Mix B to each well (standard, controls and treated cells) and mix well by pipetting up and down.

4.4 Plate measurement:

4.4.1 Measure output at OD 412 nm on a microplate reader in a kinetic mode, every 2 – 3 minutes, at 37°C protected from light, until Standard 6 (100 pmol/well) reaches OD 1.5 - 2.0.

Take an additional endpoint reading of all samples and standards.

Data Analysis:

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

- Average the duplicate readings for each standard, control and sample.
- Subtract the mean value of the blank (Standard 1) from all standard, control and sample readings. This is the corrected absorbance.
- Subtract the sample background control (cells not treated with insulin nor 2-DG) from sample reading.
- Plot the corrected absorbance values for each standard as a function of the final concentration ofV 2- DG6P.
- Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on standard curve data (use the equation that provides the most accurate fit).
- Apply the corrected sample OD reading to the standard curve to get 2-DG6P amount in the sample wells.
- Concentration of 2-DG in the test samples is calculated as:

$$2\text{-DG uptake} = (Ts/Sv) \times D = \text{pmol}/\mu\text{L} = \text{nmol/mL} = \mu\text{M}$$

Where:

Ts = amount of 2-DG6P in the sample well calculated from standard curve (pmol).

Sv = sample volume added in the sample wells (µL).

D = sample dilution factor, if sample is diluted to fit within the standard curve range