# 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.

Version 19a Last Updated: 25 June 2026

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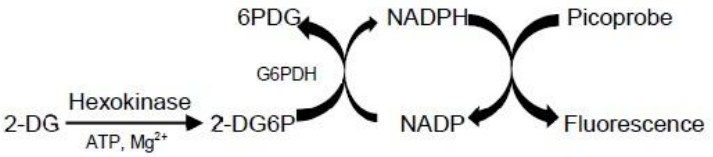
# 1. Overview

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.

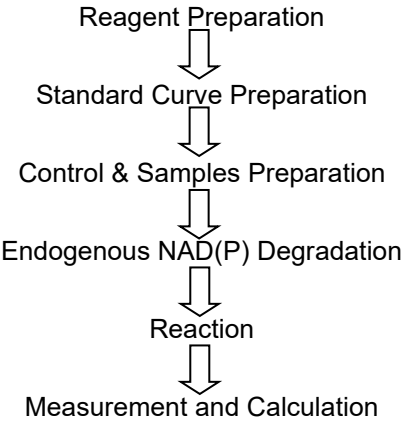
In Abcam's Glucose Uptake Assay Kit (Fluorometric), the accumulated 2-DG6P is enzymatically oxidized and coupled to a PicoProbe II, which generates fluorescence in the presence of NADPH. This easy to use non-radioactive kit can detect glucose uptake as low as 50 pmol/well and can be used for a variety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



# 3. Kits Components

| Item | Quantity |
|---|---|
| Extraction Buffer I | 17 mL |
| Neutralization Buffer V | 1 mL |
| 2-Deoxyglucose | 1 mL |
| 2-DG Uptake Assay Buffer | 10 mL |
| Enzyme Mix XXIV | 1 vial |
| PicoProbe II | 0.2 mL |
| 2-DG6P Standard | 1 vial |

# 4. Storage and Stability

Upon arrival, store the kit at -20°C and protect 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 XXIV**:

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 II:**

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 KRPH buffer and starve in 100 µl serum free adipocyte medium overnight to increase glucose uptake.

**c)** Next day, wash cells 3X with KRPH buffer.

**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 KRPH buffer.

**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 KRPH buffer and starve in 100 µl serum free adipocyte medium overnight to increase glucose uptake.

**i)** Next day, wash cells 3X with KRPH buffer.

**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-Deoxyglucose to the wells, mix by pipetting and incubate for 20 min.

**m)** Wash cells 3X KRPH buffer to remove exogenous 2-Deoxyglucose.

**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 I.

**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 V. 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 II ** | 2 µl |
| Enzyme Mix XXIV | 1 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 II ** | 2 µl x 17 = 34 µl |
| Enzyme Mix XXIV | 1 µl x 17 = 17 µl |

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

*NOTE: for samples that have 2-Deoxyglucose uptake less than **100 pmol**, repeat the assay by reducing the PicoProbe II 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-Deoxyglucose) from all sample readings

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

**d)** 2-Deoxyglucose 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.

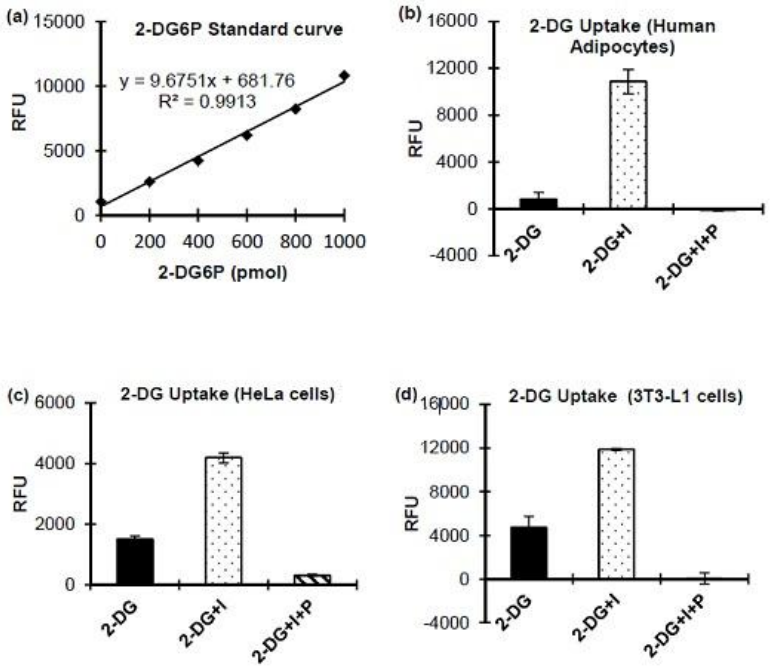



**Figure 2. (a)** 2-DG6P Standard curve **(b), (c)** and **(d)** 2-DG uptake in human adipocytes, Hela Cells and 3T3-L1 cells respectively. **I**=Insulin; **P**=Phloretin (glucose transport inhibitor). The concentration and duration of Phloretin treatment depends on the cell type and the level of expression 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. Frequently Asked Questions

**Q: I'm losing a lot of cells during the wash steps—how can I prevent this?**

Cell loss during washing is a common challenge in cell-based assays, but it can often be minimized by adjusting key aspects of the protocol:

1. Are you using the correct plate type?

Ensure that cells are seeded in a tissue culture-treated 96-well plate. Standard polystyrene plates do not support strong cell adhesion. Alternatively, use plates coated with adhesion-promoting substances such as poly-D-lysine.

Note: Protocols that simply specify a "clear 96-well plate" may not explicitly state this requirement, but it is critical for maintaining cell attachment.

2. What buffer are you using for washes?

Avoid washing cells with regular PBS that lacks divalent cations. Instead, use a buffer containing calcium and magnesium, such as:

KRH/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), or DPBS supplemented with $Ca^{2+}$ and $Mg^{2+}$

Buffers lacking these ions can promote cell detachment, increasing the risk of cell loss.

3. How many washes should be performed?

Reduce the number of washes to 1–2 instead of 3. Performing three washes can be excessive and may lead to substantial cell loss. Two washes are typically sufficient to remove residual reagents while preserving the cell monolayer.

4. Are you aspirating carefully?

Gentle aspiration is essential. For improved control, consider using a long, thin glass Pasteur pipette (with the cotton plug removed), connected to a flexible hose and vacuum trap flask. This setup allows for more precise removal of liquid without disturbing the cells.

Additional note:

These recommendations are particularly important for sensitive cell types (e.g., adipocytes), where repeated PBS washes can result in significant or complete loss of cells. Incorporating these adjustments can greatly improve assay consistency and data quality.

# 9. 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);<br>Luminescence: White plates;<br>Colorimetry: Clear plates.<br>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 |

**Technical Support**

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