Version 2b, Last updated 28 May 2024

ab234043 Glucose uptake assay (Fluorometric, Direct Glucose)

For the measurement of glucose uptake by adherent and suspension cells.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

Glucose uptake assay (Fluorometric, Direct Glucose) (ab234043) follows a simple and ultra-sensitive protocol to detect glucose uptake in various cells.

A specific hexokinase inhibitor that inhibits hexokinase, the first enzyme metabolizing glucose in cells is used to arrest glucose consumption after its uptake. Glucose Uptake is measured by using a set of enzymatic reactions that specifically oxidize glucose producing intermediates that react with the OxiRed Probe/Glucose Red Probe generating a fluorescence signal (Ex/Em=535/587 nm). The fluorescence signal is directly proportional to the amount of glucose that has been taken up and accumulated inside the cells. Unlike other kits detecting glucose derivatives, this glucose uptake assay provides a direct, powerful tool for studying this process as well as for screening and characterization of drugs that regulate glucose uptake during normal and disease development.

Seed cells in complete medium.

⇩

Starve cells (glucose- and FBS free medium) for 2-4 hours.

⇩

Incubate with glucose, FBS, Hexokinase Inhibitor and test compounds for 30 minutes.

⇩

Wash cells and then lyse cells.

⇩

Incubate lysate with OxiRed Probe/Glucose Red Probe for 30 minutes.

⇩

Measure fluorescence at Ex/Em = 535/587 nm.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer II/Assay Buffer	25 ml	-20°C	-20°C
OxiRed Probe/Glucose Red Probe (in DMSO)	200 µl	-20°C	-20°C
Development Enzyme Mix II/Enzyme Mix	1 vial	-20°C	-20°C
Hexokinase Inhibitor	1 vial	-20°C	-20°C
Glucose Standard/Glucose Standard (100 mM)	100 µl	-20°C	-20°C
Glucose Substrate/Glucose (1 M, sterile)	1 ml	-20°C	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well white plate with flat bottom.
- 24-well culture-treated plate.
- Microplate reader capable of measuring 570 nm or fluorescence at Ex/Em = 535/587 nm.
- PBS
- FBS
- Glucose-free culture media and glucose-enriched (high or low concentration) culture media.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

Warm buffers to room temperature before use.

5.1 OxiRed Probe/Glucose Red Probe

Ready to use as supplied

5.2 Development Enzyme Mix II/Enzyme Mix

1. Reconstitute with 220 µl of Assay Buffer II/Assay Buffer. Pipette up and down gently to dissolve completely.
2. Aliquot and store at -20°C. Avoid repeated freeze-thaw cycles.
3. Keep on ice whilst in use.

5.3 Hexokinase Inhibitor

1. Dissolve in 400 µl dH_2O to make 100X Hexokinase Inhibitor. Pipette up and down gently to dissolve completely.
2. Aliquot and store at -20°C. Use within 2 months. Avoid repeated freeze-thaw cycles.
3. Keep on ice whilst in use.

5.4 Glucose Substrate/Glucose (1 M, sterile)

Ready to use as supplied. Handle under sterile conditions at all times.

5.5 Glucose Standard/Glucose Standard (100 mM)

Ready to use as supplied.

5.6 Assay Buffer II/Assay Buffer

Ready to use as supplied.

5.7 Fetal Bovine Serum (user supplied)

Handle under sterile conditions at all times.

6. Standard Preparation

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

1. Dilute the Glucose Standard/100 mM Glucose Standard to 1 mM (1 nmol/µl) by adding 10 µl of 100 mM Glucose to 990 µl dH_2O, mix well.
2. Dilute the 1 nmol/µl diluted standard to 5 pmol/µl by adding 5 µl to 995 µl of dH_2O, mix well.
3. Using the 5 pmol/µl Glucose Standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Glucose Standard (µl)	Assay Buffer II/Assay Buffer (µl)	Final volume standard in well (µl)	End amount of Glucose standard in well (pmol/well)
1	0	100	50	0
2	4	96	50	10
3	8	92	50	20
4	12	88	50	30
5	16	84	50	40
6	20	80	50	50

Each dilution has enough standard to set up duplicate readings (2 x 50 µl).

7. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

The protocol below is for a **24-well tissue culture plate**. Reagents, buffers, and cell number/well should be optimized based on cell line specifications. Assay condition optimization is strongly recommended. The protocol can be scaled down for 96 wells plates.

7.1 Cell Seeding:

1. **Adherent cells:** Seed adherent cells (2-5 x 10^5) in culture media supplemented with 10% FBS one day before starting the assay. Adherent cells should be cultured to 80-90% confluence.

2. **Suspension cells:** Incubate 2-5 x 10^5 suspension cells in fresh culture media supplemented with 10% FBS the day before the assay.

7.2 Cell Starvation:

1. **Adherent cells:** After overnight incubation, remove the culture media containing 10% FBS and starve cells in glucose-free media without serum (starvation media) for 2-4 hours.

2. **Suspension cells:** Centrifuge cells at 1000 x *g* at 4°C for 5 minutes, remove the culture media, starve cells in glucose-free media without serum for 2-4 hours. After starvation, spin cells at 1000 x *g* for 5 minutes.

Δ Note: *For both adherent and suspension cells, different starvation periods may be required depending on the particular cells/cell line being used.*

7.3 Cell Treatment:

For both adherent and suspensions cells, remove the starvation medium and treat as follows.

- **Negative Control/Background:** Add 400 µl glucose-free culture media without fetal bovine serum
- **Positive Control:** Add 400 µl glucose-free media with 10 µl of Glucose Substrate/1 M Glucose, 40 µl of 10% Fetal Bovine Serum, and 4 µl of 100X Hexokinase Inhibitor.
- **Compound Testing:** Add 400 µl glucose-free media with test compounds with 10 µl of Glucose Substrate/1 M Glucose and 4 µl of 100X Hexokinase Inhibitor.

For all treatments, incubate the cells at 37°C with 5% CO_2 for 30 minutes.

7.4 Cell Lysis:

1. **Adherent cells:** Remove the media, wash twice with 500 µl ice-cold 1X PBS with 1X Hexokinase Inhibitor, and then lyse cells with 400 µl Assay Buffer II/Assay Buffer/1X Hexokinase Inhibitor (i.e. 396 µl Assay Buffer II/Assay Buffer + 4 µl 100X Hexokinase Inhibitor). Place the plate on a shaker (medium speed) to allow lysis for 10 minutes.

2. **Suspension cells:** Pellet cells at 1000 x *g* for 5 minutes. Remove the media, wash cells twice with 500 µl ice-cold 1X PBS with 1X Hexokinase Inhibitor. Pellet cells (1000 x *g*; 5 minutes) and remove PBS. Repeat this step once. Lyse cells with 400 µl Assay Buffer II/Assay Buffer/1X Hexokinase Inhibitor.
   Place the plate on a shaker (medium speed) to allow lysis for 10 minutes.

For both adherent and suspension cells, the lysates should be treated as follows.

- Transfer the lysates to 1.5 ml Eppendorf tubes, and centrifuge at 12000 x *g* for 5 minutes. Save the supernatants.
- Add 5-20 µl of sample supernatant (about 0.5-2 µg protein) into a 96-well white plate with flat bottom and bring the volume to 50 µl with Assay Buffer II/Assay Buffer/1X Hexokinase Inhibitor.

Δ Note: *For unknown samples, it is recommended to test several volumes of your samples to ensure the readings are within the standard curve range.*

7.5 Reaction Mix:

Dilute the OxiRed Probe/Glucose Red Probe 100-fold (i.e. 2 µl OxiRed Probe/Glucose Red Probe + 198 µl DMSO).

Δ Note: *Always use freshly diluted OxiRed Probe/Glucose Red Probe.*

Component	Reaction Mix (µl)
Assay Buffer II/Assay Buffer	47
Diluted OxiRed Probe/Glucose Red Probe	1
Development Enzyme Mix II/Enzyme Mix	2

1. Add 50 µl of Reaction Mix into each standard, sample and test well.
2. Mix well.
3. Incubate for 30 minutes at 37°C protected from light.

7.6 Measurement:

Measure the fluorescence at Ex/Em=535/587 nm in a microplate reader.

8. 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.

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (zero glucose) from all readings.
3. If significant, subtract the negative control from sample readings.
4. Plot the corrected values for each standard as a function of the amount of glucose to generate a standard curve.
5. Apply the corrected sample readings to the standard curve to get the amount of glucose in the sample wells.
6. Concentration of glucose in nmol/ml in the test samples is calculated as:

$$\text{Glucose concentration} = \frac{B}{V} * D$$

Where:

B = amount of glucose in the sample well calculated from standard curve in pmol.

V = sample volume added in the sample wells in µl.

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

9. Typical Data

Data provided **for demonstration purposes only.**

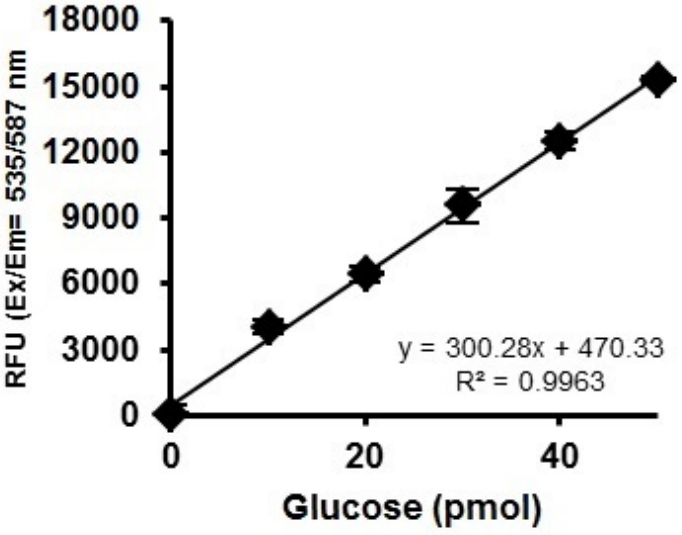

Figure 1. Glucose standard curve.

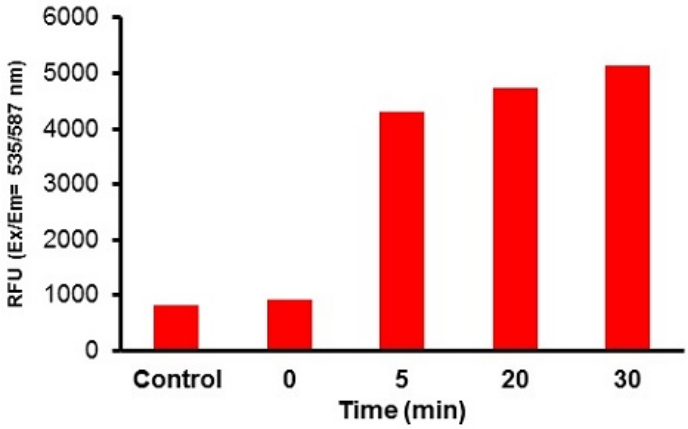

Figure 2: Glucose uptake time course. Jurkat (Human T cell leukemia cell line from peripheral blood) cells were starved (glucose-free, FBS-free medium). Inhibitor incubation time, 2 hours.

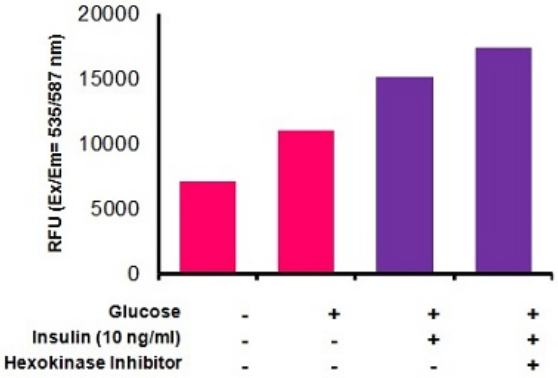

Figure 3: NIH/3T3 (Mouse embryo fibroblast cell line) cells were starved (glucose-free and FBS-free) for 24 hours then switched to glucose-containing medium and treated +/- insulin (10 ng/ ml) for 15 minutes in the presence or absence of Hexokinase Inhibitor.

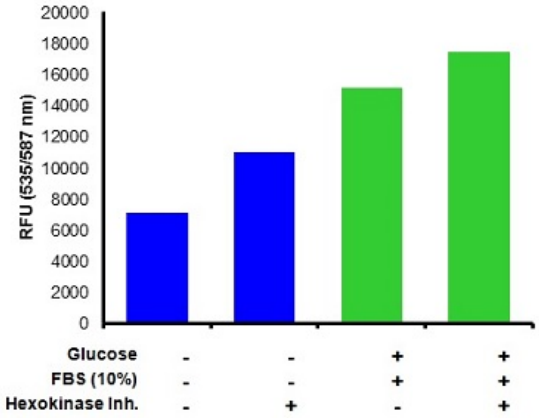

Figure 4: HeLa (Human epithelial cell line from cervix adenocarcinoma) cells were starved for 2 hours (glucose-free and FBS-free media) then switched to either glucose- and FBS-free medium or complete (with 10% FBS) medium with or without Hexokinase Inhibitor for 30 minutes.

10. Notes

Technical Support

Copyright © 2024 Abcam. All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)

Copyright © 2024 Abcam. All rights reserved