Cell Meter™ Glucose Uptake Imaging Kit *Red Fluorescence*

Catalog number: 23501
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 23501)
Component A: Glutite™ Red 670	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (25 mL)
Component C: DMSO	Refrigerated (2-8 °C)	1 vial (100 µL)
Component D: 5X KRPH Buffer	Freeze (< -15 °C), Minimize light exposure	1 vial (20 mL)

OVERVIEW

Glucose metabolism is a primary source of cellular energy and biomaterials for maintaining cell homeostasis. Surplus glucose is stored as glycogen in muscular and hepatic tissues, with subsequent release into the bloodstream as needed. The GLUT family of transporter proteins facilitates the process of glucose uptake under the regulation of multiple mechanisms, including the modulation by hormones and growth factors like insulin. Notably, cancer cells exhibit a marked propensity for increased glucose uptake and metabolic activity, engaging in aerobic glycolysis to sustain their accelerated proliferation rates. Compounds that hinder glucose uptake in cancer cells exhibit discernible anti-cancer effects. The Cell Meter™ Glucose Uptake Imaging Kit provides a simple and direct method for quantifying glucose uptake across diverse cellular contexts. This kit employs Glutite™ Red 670, a cell-permeable fluorescent glucose tracer, enabling precise molecular sensing and bioimaging via GLUT transporters. The robust red fluorescence emitted by Glutite™ Red 670 (Ex/Em = 651/670 nm) correlates proportionally with cellular glucose uptake, offering a valuable means for quantification via both fluorescence microscopy and flow cytometry techniques.

AT A GLANCE

Important

Thaw all the components at room temperature before starting the experiment.

Protocol Summary

1. Prepare cells with your test compounds.
2. Add Glutite™ Red 670 dye working solution.
3. Incubate cells at 37 °C for 30 to 60 minutes.
4. Remove Glutite™ Red 670 dye working solution.
5. Wash cells with PBS.
6. Analyze cells using a fluorescence microscope with a Cy5 filter.

KEY PARAMETERS

Fluorescence microscope

Emission	Cy5 Filter Set
Excitation	Cy5 Filter Set
Recommended plate	Black wall/clear bottom

CELL PREPARATION

Prepare Differentiated 3T3-L1 Adipocytes

1. Grow 3T3-L1 fibroblasts 2 days post-confluence in a 75 cm flask using DMEM supplemented with 10% FBS.
2. To initiate differentiation of 3T3-L1 cells, remove the medium and add DMEM supplemented with 10% FBS, 0.83 µM insulin, 0.25 µM dexamethasone, and 0.25 mM isobutylmethylxanthine. Incubate for 2 days.
3. Remove the medium and add DMEM supplemented with 10% FBS and 0.83 µM insulin. Incubate for 2 days.
4. Remove the medium and add DMEM supplemented with 10% FBS. Incubate for 3-5 days.
5. Differentiated cells (at least 95% of which showed an adipocyte phenotype by the accumulation of lipid droplets) were used on days 8 to 12 after induction of differentiation.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Glutite™ Red 670 Stock Solution (100X)

1. Add 100 µL of DMSO into Glutite™ Red 670 vial (Component A) to make a stock solution.

   Note: Any unused Glutite™ Red 670 stock solution should be divided into single-use aliquots and stored at ≤ -20 ° C. Protect from light and avoid repeated freeze-thaw cycles.

PREPARATION OF WORKING SOLUTION

Glutite™ Red 670 Working Solution

1. Prepare the working solution by adding 100 µL of Glutite™ Red 670 stock solution into 10 mL of Assay Buffer (Component B).

   Note: Protect the working solution from light by covering it with foil or placing it in the dark.

   Note: For best results, this solution should be used within a few hours of its preparation.

   Note: 10 mL of working solution is enough for 100 tests.

1X KRPH Buffer

1. Add 20 mL of 5X KRPH buffer (Component D) to 80 mL of deionized water.

   Note: 50 mL volume of 1X KRPH buffer is enough for approximately one 96-well plate. Prepare the needed volume proportionally. Store any unused 1X KRPH buffer at 4ºC or -20 ºC.

SAMPLE EXPERIMENTAL PROTOCOL

Important

The following protocol serves as guidelines to culture 3T3-L1adipocytes for Glutite™ Red 670 uptake in a 96-well plate, and should be modified according to your specific needs.

1. Plate the entire 75 cm flask of 3T3-L1 adipocytes, 100 µL/well, in a 96-well black wall/clear bottom cell culture Poly-D lysine plate for 4-6 hours before the experiment.
2. Remove the cell plate from the incubator, aspirate the medium from the wells, and deprive the cells with 100 µL/well/ 96 well-plate serum-free medium. Incubate the cells at 37 °C, 5% CO_2 for 6 hours to overnight.
3. Remove the cell plate from the incubator, aspirate the medium from the wells, and gently wash the cells twice with 100 µL/well 1X KRPH buffer.
4. Add 90 µL/well of Assay Buffer (Component B) and incubate the cells at 37 °C, 5% CO_2 incubator for 1 hour.
5. Treat cells with insulin, without insulin, or with the test compound for 2 hours. Add 10 µL/well of the 10X insulin solution for a final concentration of 1 µM or add 10 µL/well of the 10X test compound solution. Additionally, add 10 µL of insulin vehicle buffer or compound vehicle buffer to the untreated wells as control, and incubate at 37 °C, 5% CO_2 for 60 minutes.
6. For the glucose uptake inhibition study, add 10X Phloretin to a final concentration of 200 µM or test inhibitors, and incubate at 37 °C, 5% CO_2 for 60 minutes.

   Note: It is recommended to add 10 µL of inhibitor vehicle buffer to both the insulin-treated and untreated wells as a control.
7. Add 100 µL/well of Glutite™ Red 670 dye working solution, and incubate for 60 minutes.

   Note: Optimal incubation time will need to be determined for each cell line and experiment
8. Remove Glutite™ Red 670 dye staining solution without disturbing the cells.
9. Wash the cells with PBS twice.
10. Keep the cells in 100 µL/well (96-well plate) of Assay Buffer (Component B).
11. Monitor the fluorescence signal using a fluorescence microscope with a Cy5 filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

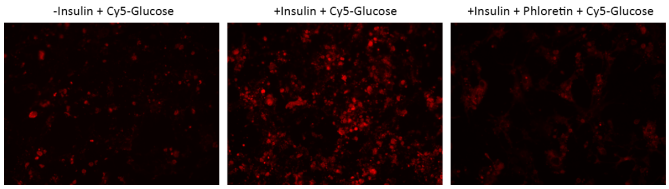

Figure 1. Fluorescence images of Glutite™ Red 670 uptake in differentiated 3T3-L1adipocytes using Cell Meter™ Glucose Uptake Imaging Kit. Differentiated 3T3-L1 cells at 50,000 cells/wells/100 µL were seeded overnight in a 96-well Poly-D-Lysine black wall/clear bottom plate. The cells were pre-treated with insulin for 2 hours before being treated with Glutite™ Red 670 and phloretin for 60 minutes. The images were acquired using a fluorescence microscope with a Cy5 filter set.

DISCLAIMER