Screen Quest™ Fluorimetric Glucose Uptake Assay Kit

Catalog number: 36500, 36501
Unit size: 100 Tests, 500 Tests

Component	Storage	Amount	
		Cat No. 36500	Cat No. 36501
Component A: 2-Deoxyglucose (2-DG, 10mM)	Freeze (<-15 °C), Minimize light exposure	1 mL	5 mL
Component B: Glucose Uptake Buffer	Freeze (<-15 °C), Minimize light exposure	10 mL	50 mL
Component C: Acidic Lysis Buffer	Freeze (<-15 °C), Minimize light exposure	2.5 mL	12.5 mL
Component D: Neutralization Buffer	Freeze (<-15 °C), Minimize light exposure	2.5 mL	12.5 mL
Component E: Enzyme Probe	Freeze (<-15 °C), Minimize light exposure	1 bottle (lyophilized powder)	5 bottles (lyophilized powder)
Component F: Assay Buffer	Freeze (<-15 °C)	1 bottle (5 mL)	1 bottle (25 mL)
Component G: NADP	Freeze (<-15 °C), Minimize light exposure	1 vial	5 vials
Component H: 5x KRPH Buffer	Freeze (<-15 °C), Minimize light exposure	20 mL	100 mL

OVERVIEW

Glucose transport systems are responsible for transporting glucose across cell membranes. Measuring uptake of 2-deoxyglucose (2-DG), a glucose analog, in tissues and cells is widely accepted as a reliable method to estimate the amount of glucose uptake and to investigate the regulation of glucose metabolism and mechanism of insulin resistance. The 2-DG uptake is commonly determined by using non-metabolized 2-DG labeled with tritium or C14. However, routine use of a radiolabelled probe is costly and requires a tedious special handling procedure. AAT Bioquest's Screen Quest™ Fluorimetric Glucose Uptake Assay Kit provides a sensitive and non-radioactive assay in cells. In this assay 2-DG is taken up by glucose transporters, and metabolized to 2-DG-6-phosphate (2-DG6P). The non-metabolizable 2-DG6P accumulates in the cells, and is proportional to glucose uptake by cells. The accumulated 2-DG6P is enzymatically oxidized and generates NADPH, which is specifically monitored by a fluorogenic NADPH sensor. The signal can be read by a fluorescence microplate reader.

AT A GLANCE

Protocol summary

1. Plate cells and treat the cells as desired
2. Add 10 µL/well 2-DG and incubate at 37°C for 20-40 minutes
3. Wash cells and lyse cells
4. Add 50 µL/well of 2-DG Uptake Assay working solution
5. Incubate at RT for 30 to 120 minutes
6. Monitor fluorescence increase at Ex/Em = 540/590 nm

Important Thaw all the kit components at room temperature before starting the experiment. The above mentioned protocol is for one 96-well plate.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

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.

KRPH Buffer stock solution (1X):
Add 20 mL of KRPH Buffer (5X) (Component H) to 80 mL of deionized water and mix them well.

Note 50 mL volume of 1× KRPH Buffer is enough for approximately one 96-well plate. Prepare the needed volume proportionally. Store the unused 1×KRPH at 4°C or -20°C.

PREPARATION OF WORKING SOLUTION

1. NADP working solution:
Add 100 µL of H_2O into the vial of NADP (Component G) and mix them well.

2. Enzyme Probe working solution:
Add 5 mL of Assay Buffer (Component F) into the bottle of Enzyme Probe (Component E) and mix them well.

3. 2-DG Uptake Assay working solution:
Add 100 µL of NADP working solution into the Enzyme Probe working solution and mix them well.

Note These quantities are good for one 96-well plate.

SAMPLE EXPERIMENTAL PROTOCOL

Important: This protocol can be used as guidelines to culture 3T3-L1 adipocytes for 2-DG uptake.

1. Plate cells in growth medium at 50,000-80,000 cells/well/100 µL/96-well or 12,500-20,000 cells/well/25 µL/384-well black wall/clear bottom cell culture Poly-D lysine plate for 4-6 hours before 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) or 25 µL/well (384 well-plate) serum free medium. Incubate the cells at 37°C, 5% CO_2 incubator 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 1× KRPH buffer.

4. Add 90 µL/well Glucose Uptake Buffer (Component B) and incubate the cells at 37°C, 5% CO_2 incubator for 1 hour.

5. Stimulate with or without insulin or compound of test for 20 mins. Add 10 µL/well of the 10× insulin solution to a final concentration of 1 µM or 10× compound solution of test. And also add 10 µL insulin vehicle buffer or compound vehicle buffer to the untreated wells as control, and incubate at 37°C, 5% CO_2 incubator for 20 mins.

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6. For glucose uptake inhibition study, add 10× Phloretin to a final concentration of 200 uM or inhibitors of test, and incubate at 37°C, 5% CO_2 for 2-5 min.

   Note 10 mL inhibitor vehicle buffer is suggested to be added to both the insulin treated and untreated wells as control. Phloretin treated cells can be used as positive control.

7. Add 10 µL/well 2-DG solution (Component A) to each well, and incubate at 37°C, 5% CO_2 incubator for 20-40 min. For negative controls, leave some wells untreated with insulin, inhibitor and 2-DG.

8. After treatment, remove solution in each well and gently wash cells 3 times, 100 µL/well with KRPH Buffer to remove the extra 2-DG from the solution. Remove KRPH Buffer from the wells.

9. Add 25 µL/well Acidic Lysis Buffer (Component C) to each well and incubate at 37°C for 20 mins to lyse the cells. And the 2DG uptake assay mixture could be prepared in the meantime.

10. Add 25 µL/well Neutralization Buffer (Component D) to each well, mix thoroughly, leave at room temperature for 5-10 minutes to neutralize the cell lysate.

11. Add 50 µL of 2DG Uptake Assay working solution to each well of 2DG6P standard (Not provided) or cell lysate.

12. Incubate the reaction at room temperature for 30 minutes to 2 hours, protected from light.

13. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 530-570/590-600 nm (optimal Ex/Em = 540/590 nm, cut off at 570 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

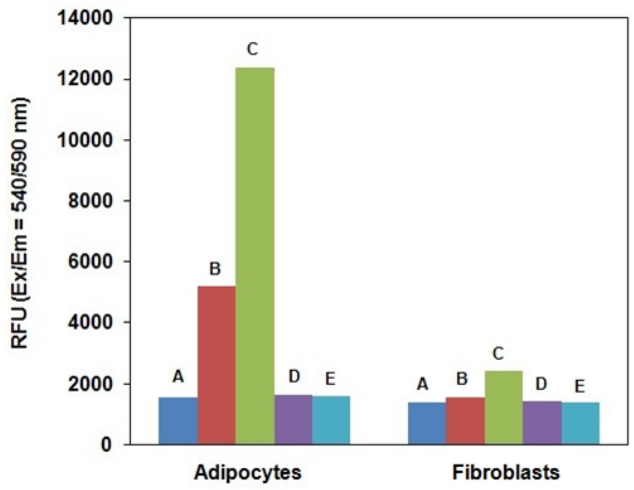

Figure 1. Measurement of 2DG uptake in differentiated 3T3-L1 adipocytes and 3T3-L1 fibroblasts. Assays were performed with Screen Quest™ Fluorimetric Glucose Uptake Assay Kit in a black wall/clear bottom cell culture Poly-D lysine plate using a Gemini (Molecular Devices) microplate reader. (A: Negative Control, no insulin no 2-DG treatment. B: 2DG uptake in the absence of insulin. C: 2DG uptake in the presence of 1mM insulin. D: 2DG uptake in the presence of 1mM insulin and 200 mM phloretin. E: 2DG uptake in the presence of insulin 1mM and 5mM D-Glucose.)

DISCLAIMER