

Screen Quest™ Colorimetric Glucose Uptake Assay Kit

Catalog number: 36503, 36504
Unit size: 100 Tests, 500 Tests

Component	Storage	Amount (Cat No. 36503)	Amount (Cat No. 36504)
Component A: 2-Deoxyglucose	Freeze (< -15 °C), Minimize light exposure		1 bottle (5 mL)
Component A: 2-Deoxyglucose (2-DG, 10mM)	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mL)	
Component B: Glucose Uptake Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (10 mL)	1 bottle (50 mL)
Component C: Acidic Lysis Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (2.5 mL)	1 bottle (12.5 mL)
Component D: Neutralization Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (2.5 mL)	1 bottle (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 KRPB Buffer	Freeze (< -15 °C), Minimize light exposure		1 bottle (100 mL)
Component H: 5x KRPB Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (20 mL)	

OVERVIEW

Screen Quest™ Colorimetric Glucose Uptake Assay Kit is designed for measuring glucose uptake in tissues or cultured cells using a non-radioactive colorimetric assay format. Glucose transport systems move glucose across cell membranes, and uptake of 2-deoxyglucose (2-DG), a glucose analog, is widely used to estimate cellular glucose uptake and study the regulation of glucose metabolism. In this assay, 2-DG is taken up by glucose transporters and converted to 2-deoxyglucose-6-phosphate (2-DG6P), which accumulates inside cells because it is not further metabolized through glycolysis. The accumulated 2-DG6P is enzymatically oxidized to generate NADPH, which is detected using a chromogenic NADPH sensor. The colorimetric signal is measured with an absorbance microplate reader using the OD ratio at 570 nm to 610 nm. Screen Quest™ Colorimetric Glucose Uptake Assay Kit is suitable for glucose uptake analysis, glucose transporter studies, metabolic regulation research, and insulin resistance-related cell or tissue studies.

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 OD ratio increase at 570/610 nm

Important Note

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

KEY PARAMETERS

Absorbance microplate reader

Absorbance	570/610 nm
Recommended plate	Clear bottom

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

KRPB Buffer stock solution (1X)

Add 20 mL of KRPB Buffer (5X) (Component H) to 80 mL of deionized water and mix them well.

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

PREPARATION OF WORKING SOLUTION

NADP working solution

Add 100 µL of H₂O into the vial of NADP (Component G) and mix them well.

Enzyme Probe working solution

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

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% CO₂ 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× KRPB buffer.
4. Add 90 µL/well Glucose Uptake Buffer (Component B) and incubate the cells at 37°C, 5% CO₂ incubator for 1 hour.
5. Stimulate with or without insulin or compound of test for 20 min. 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% CO₂ incubator for 20 mins.

- 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% CO₂ for 2-5 mins.

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.

- Add 10 µL/well 2-DG solution (Component A) to each well, and incubate at 37°C, 5% CO₂ incubator for 20-40 mins. For negative controls, leave some wells untreated with insulin, inhibitor and 2-DG.
- 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.
- Add 25 µL/well Acidic Lysis Buffer (Component C) to each well and incubate at 37°C for 20 min to lyse the cells. And the 2DG uptake assay mixture could be prepared in the meantime.
- 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.
- Add 50 µL of 2DG Uptake Assay working solution to each well of 2-DG6P standard (Not provided) or cell lysate.
- Incubate the reaction at room temperature for 30 minutes to 2 hours, protected from light.
- Monitor the absorbance ratio increase at 570/610 nm with an absorbance plate reader.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.

EXAMPLE DATA ANALYSIS AND FIGURES

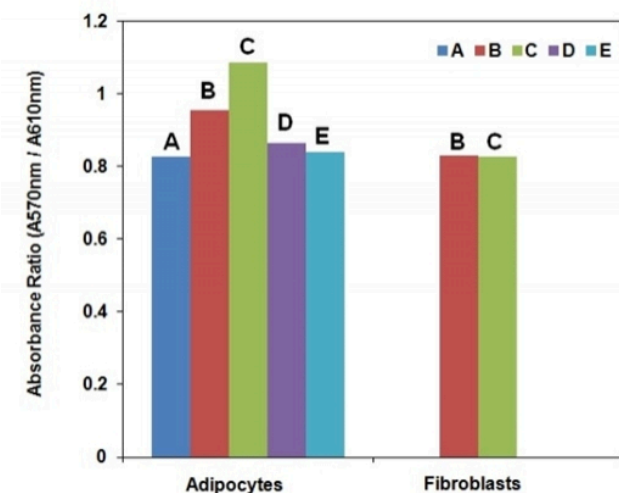


Figure 1. Measurement of 2DG uptake in differentiated 3T3-L1 adipocytes and 3T3-L1 fibroblasts. Assays were performed with Screen Quest™ Colorimetric Glucose Uptake Assay Kit in a black wall/clear bottom cell culture Poly-D lysine plate using a SpectraMax (Molecular Devices) microplate reader. (A: Negative 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.)