Screen Quest™ Colorimetric Glucose Uptake Assay Kit

Blue Color

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 36503 (100 assays) 36504 (500 assays)	Keep in freezer Avoid exposure to light	Absorbance microplate readers

Introduction

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 C^{14}. However, the routine use of a radiolabelled probe is costly and requires a tedious special handling procedure.

AAT Bioquest's Screen Quest™ Colorimetric Glucose Uptake Assay Kit provides a sensitive and non-radioactive assay for measuring glucose uptake in cultured cells. In this assay, 2-DG is taken up by glucose transporters, and metabolized to 2-DG-6-phosphate (2-DG6P). The amount of the accumulated non-metabolizable 2-DG6P is proportional to glucose uptake by cells. In this assay, the accumulated 2-DG6P is enzymatically coupled to generate NADPH, which is specifically monitored by a NADPH sensor. The signal can be read by an absorbence microplate reader by reading the OD ratio at wavelength 570 to 610 nm.

Kit Components

Components	**Amount**	
	Cat# 36503 (100 assays)	**Cat# 36504 (500 assays)**
Component A: 2-Deoxyglucose (2-DG, 10mM)	1 mL	5 mL
Component B: Glucose Uptake Buffer	10 mL	50 mL
Component C: Acidic Lysis Buffer	2.5 mL	12.5 mL
Component D: Neutralization Buffer	2.5 mL	12.5 mL
Component E: Enzyme Probe	1 bottle (lyophilized powder)	5 bottles (lyophilized powder)
Component F: Assay Buffer	1 bottle (5 mL)	1 bottle (25 mL)
Component G: NADP	1 vial	5 vials
Component H: 5× KRPH Buffer	20 mL	100 mL

Assay Protocol for One 96-Well Plate

Brief Summary

Plate cells and treat the cells as desired→ Add 2-DG and incubate at 37°C for 20-40 min → Wash cells and lyse cells → Add 100µL/well of Assay Mixture→ incubate at RT for 30 to 120 min → Monitor OD ratio increase at 570/610 nm

1. **Prepare Cells:**

   The following protocols are guidelines to culture 3T3-L1adipocytes for 2-DG uptake.

   1.1 Prepare differentiated 3T3-L1 adipocytes: 3T3-L1 fibroblasts were grown 2 days post-confluence in a 75 cm flask with DMEM supplemented with 10% FBS. For induction of differentiation of 3T3-L1 preadipocytes into mature adipocytes, the cells were incubated 2 days with DMEM supplemented with

10% FBS, 0.83µM insulin, 0.25µM dexamethasone, and 0.25mM isobutylmethylxanthine. The cells were maintained for 2 days with DMEM supplemented with 10% FBS and 0.83µM insulin alone. The medium was changed to DMEM supplemented with 10% FBS for another 3-5 days. Differentiated cells (at least 95% of which showed an adipocyte phenotype by accumulation of lipid droplets) were used on day 8 to 12 after induction of differentiation.

1.2 Plate 3T3-L1 adipocytes in growth medium at 50,000-80,000 cells/well/100µL/96-well or 12,500-20,000 cells/well/25µL/384-well black wall/clear bottom cell culture Poly-D lysine plate for 4-6 hours before experiment.

1.3 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 ºC, 5% CO_2 incubator for 6 hours to overnight.

2. Treat Cells:

2.1 Prepare 1× KRPH buffer: Add 20 mL of 5×KRPH Buffer (Component H) to 80 mL of deionized water.

*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*ºC or *-20 ºC.*

2.2 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.

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

2.4 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 ºC, 5% CO_2 incubator for 20 min.

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

Note: 10µL inhibitor vehicle buffer is suggested to be added to both the insulin treated and untreated wells as control.

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

Table 1. An example layout of experiment in a 96-well-plate

1	**3**	**5**									
1	**3**	**5**									
1	**3**	**5**									
1	**3**	**5**									
2	**4**	**6**									
2	**4**	**6**									
2	**4**	**6**									
2	**4**	**6**									

Table 2. Treatment condition examples

1	*- insulin + 2-DG*	*4*	*+ insulin -Phloretin + 2DG*
2	*+ Insulin + 2DG*	*5*	*+Insulin + Glucose (5mM) + 2DG*
3	*+Insulin + Phloretin + 2DG*	*6*	*- Insulin - 2DG*

-Insulin: only add insulin vehicle buffer; -Phloretin: only add Phloretin vehicle buffer; -2DG: only add H_2O

3. Lyse Cells:

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

3.2 Add 25 µL/well Acidic Lysis Buffer (Component C) to each well and incubate at 37 ºC for 20 min to lyse the cells. And the 2DG uptake assay mixture could be prepared in the meantime (see Step 4).
3.3 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.

4. Run glucose uptake assay:

4.1 Add 100 µL of H_2O into the vial of NADP (Component G) to reconstitute NADP.
4.2 Add 5 mL of Assay Buffer (Component F) into the bottle of Enzyme Probe (Component E).
4.3 Add 100 µL reconstituted NADP solution (from Step 4.1) into the bottle of Component E (from Step 4.2) to make the 2DG uptake assay mixture.
4.4 Add 50 µL of 2DG uptake assay mixture (from Step 4.3) to each well of 2DG6P standard or cell lysate.
4.5 Incubate the reaction at room temperature for 30 minutes to 2 hours, protected from light.
4.6 Monitor the absorbance ratio increase at 570/610 nm with an absorbance plate reader.

Data Analysis

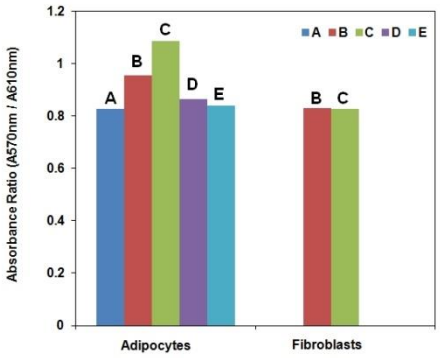

Figure 1. Measurement of 2DG uptake in differentiated 3T3-L1 adipocytes and 3T3-L1 fibroblasts. Assays were performed with Screen Quest™ Colorimetric Glucose Uptake Assay Kit in a black wall/clear bottom cell culture Poly-D lysine plate using a SpectraMax (Molecular Devices) microplate reader. (A: Negative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

References

1. Takala, T. E., and ILMO E. Hassinen. "Effect of mechanical work load on the transmural distribution of glucose uptake in the isolated perfused rat heart, studied by regional deoxyglucose trapping." *Circulation Research* 49.1 (1981): 62-69.
2. Durham, D. I. A. N. N. E., THOMAS A. Woolsey, and L. A. W. R. E. N. C. E. Kruger. "Cellular localization of 2-[3H] deoxy-D-glucose from paraffin-embedded brains." *The Journal of Neuroscience* 1.5 (1981): 519-526.
3. Vannucci, Robert C., and Susan J. Vannucci. "Glucose metabolism in the developing brain." *Seminars in perinatology*. Vol. 24. No. 2. WB Saunders, 2000.
4. Duncan, Gary E., Walter E. Stumpf, and Christof Pilgrim. "Cerebral metabolic mapping at the cellular level with dry-mount autoradiography of [^{3}H] 2-deoxyglucose." *Brain research* 401.1 (1987): 43-49.