Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

EZCell™ Direct Glucose Uptake Assay Kit

Rev 04/19

(Catalog # K924-50; 50 assays; Store at -20°C)

I. Introduction:

Glucose uptake is one of the key processes for cellular glucose metabolism. The study of glucose uptake can provide important information for understanding glucose metabolism and regulation in normal and disease development such as diabetes. BioVision's EZCell™ Glucose Uptake Assay kit is simple, ultra-sensitive and easy to use. 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 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.

D-Glucose + OxiRed™ Probe $\xrightarrow{\text{Enzyme Mix/Hexokinase Inhibitor}}$ High/Low Fluorescence (Ex/Em =535/587 nm)

II. Application:

- Measurement of glucose uptake in various cells
- Analysis of cell signaling that regulate glucose uptake in various cells
- Study and characterize stimuli/inhibitors of glucose uptake

III. Sample Type:

- Adherent or suspension cells

IV. Kit Contents:

Components	K924-50	Cap Code	Part Number
Assay Buffer	25 ml	WM	K924-50-1
OxiRed™ Probe (in DMSO)	200 µl	Red	K924-50-2
Enzyme Mix	1 vial	Green	K924-50-3
Hexokinase Inhibitor	1 vial	Orange	K924-50-4
Glucose Standard (100 mM)	100 µl	Yellow	K924-50-5
Glucose (1 M, Sterile)	1 ml	Blue	K924-50-6

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom and 24 well culture-treated plate
- Microplate reader (ELISA plate reader) capable of reading fluorescence
- PBS, FBS
- Glucose-Free Culture Media and Glucose-Enriched (High or Low Concentration) Culture Media

VI. Reagent Preparation and Storage Conditions:

Store kit at -20°C, protected from light. Warm assay buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

- **OxiRed™ Probe (in DMSO), Glucose Standard (100 mM):** Ready to use as supplied. Warm to room temperature before use. **Enzyme Mix**: Reconstitute with 220 µl Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use.
- **Hexokinase Inhibitor**: Dissolve with 400 µl dH_2O to make 100X Hexokinase Inhibitor. Reconstitute as needed. Pipette up and down to dissolve completely. Put on ice while in use. Aliquot and store at -20°C. Use within two months.
- **Fetal Bovine Serum and Glucose (1 M, Sterile)**: Both reagents should be should be handled under *sterile conditions at all times*.

VII. Glucose Uptake Assay Protocol:

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. Protocol can be scaled down for 96 wells plates.

1. **Cell seeding:** For 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. For suspension cells: incubate 2-5 x 10^5 suspension cells in fresh culture media supplemented with 10% FBS the day before the assay.
2. **Starvation:** For adherent cells: After overnight incubation, remove the culture media with 10% FBS and starve cells in Glucose-free media without serum (starvation media) for 2-4 hrs*. For suspension cells: spin down 1000 x g at 4°C for 5 min, remove the culture media and starve cells in Glucose-free media without serum for 2-4 hrs*. After starvation, spin cells at 1000 x g for 5 min.

   ***Notes:** Different cell types may require different starvation times.
3. **Treatment:** For adherent/suspension cells: remove the starvation media and treat cells as follows: (a) 400 µl Glucose-free culture media without fetal bovine serum (Negative Control/Background), (b) 400 µl Glucose-free media with 10 µl of 1 M Glucose, 40 µl of 10X Fetal Bovine Serum, and 4 µl of 100X Hexokinase Inhibitor (Positive Control), or (c) 400 µl Glucose-free media with test compounds with 10 µl of 1 M Glucose and 4 µl of 100X Hexokinase Inhibitor. Incubate the cells at 37°C with 5% CO_2 for 30 min.

4. **Cell Lysis:** For 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/1X Hexokinase Inhibitor (i.e. 396 µl Assay Buffer + 4 µl 100X Hexokinase Inhibitor). Place the plate on a shaker (medium speed) to allow lysis for 10 minutes. For suspension cells: spin down 1000 x g for 5 mins. Remove the media, wash cells twice with 500 µl ice-cold 1X PBS with 1X Hexokinase Inhibitor. Spin cells down (1000 x g; 5 min) and remove PBS. Repeat this step once. Lyse cells with 400 µl Assay Buffer/1X Hexokinase Inhibitor: Place the plate on a shaker (medium speed) to allow lysis for 10 minutes. Transfer the lysates to 1.5 ml Eppendorf tubes, and then spin down at 12000 x g for 5 min. 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/1X Hexokinase Inhibitor. **Notes:** For unknown samples, we recommend testing several volumes of your samples to ensure the readings are within the standard curve range.
5. **Standard Curve Preparation:** Dilute the 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. Dilute the 1 nmol/µl diluted standard to 5 pmol/µl by adding 5 µl to 995 µl of dH_2O, mix well. Add 0, 2, 4, 6, 8, 10 µl of the 5 pmol/µl glucose into a 96-well white plate with flat bottom to generate 0, 10, 20, 30, 40, and 50 pmol/well standards. Adjust volume to 50 µl per well with Assay Buffer.
6. **Reaction Mix:** Dilute OxiRed™ Probe 100-fold (i.e. 2 µl Probe + 198 µl DMSO). Mix enough reagents for the number of assays (samples and standards) to be performed. For each well, prepare 50 µl Reaction Mix containing:

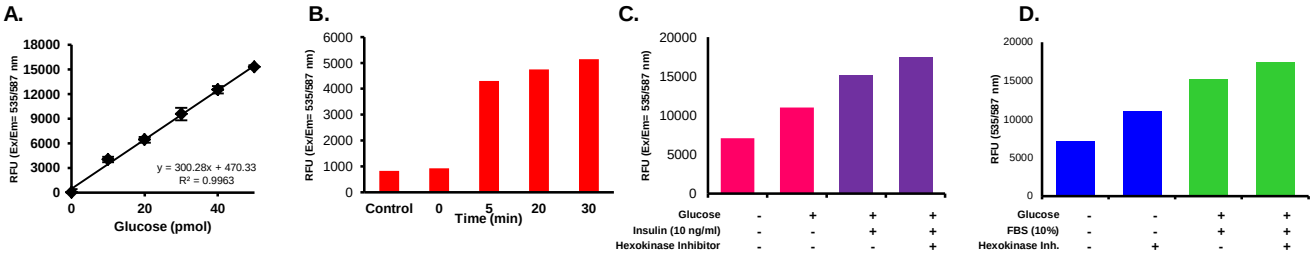
	Reaction Mix
Assay Buffer	47 µl
Diluted OxiRed™ Probe	1 µl
Enzyme Mix	2 µl

Add 50 µl of the Reaction Mix to each well containing the Standard, test samples and negative and positive control wells, mix well. Incubate the reaction for 30 minutes at 37°C and protect from light.
Note: Discard unused diluted OxiRed™ Probe. Always use freshly diluted probe.

7. **Measurement:** Measure the fluorescence at Ex/Em=535/587 nm in a micro plate reader.
8. **Calculation:** Subtract the 0 Glucose blank reading from all readings. If negative control reading is significant, subtract the negative control reading from samples. Plot the standard curve. Apply the corrected sample reading to the standard curve to get Glucose amount in the sample wells.

Glucose concentration in the sample can be calculated as follows:

C= B/V x Dilution Factor = pmol/µl = nmol/ml *(Glucose in sample can also be expressed in pmol/well of sample)*
Where: **B** is the amount of glucose in the sample (pmol).
V is the sample volume used in the reaction well (µl).
Glucose molecular weight: 180.2 g/mole.



Figures. **A)** Glucose standard curve. **B)** Glucose Uptake time course, Jurkat Cells: Cells were starved (Glucose-free, FBS-free media, inhibitor incubation time: 2 h. **C)** 3T3-L1 cells were Glucose and FBS Deprived for 24 hrs, switched to media with Glucose, stimulated without or with Insulin (10 ng/ml) for 15 min (green bars), with or without 1X Inhibitor (pink bars) **D)**. HeLa cells were Glucose and FBS deprived for 2 hr, then switched to Glucose and FBS-free media (Control), or complete media (10% FBS) with or without 1X Inhibitor for 30 min (Blue bars: no inhitor; maroon bars: with inhibitor).

VIII. RELATED PRODUCTS:

Glucose Uptake Fluorometric Assay (K666)
Glucose Assay Kit II (K686)
Maltose and Glucose Assay Kit (K618)
NADP/NADPH Quantification Kit (K347)
Glucose Dehydrogenase Activity Assay Kit (K786)
Glucose-6-Phosphate Dehydrogenase Assay Kit (K757)
Glucose Uptake Colorimetric Assay Kit (K676)
Lactate Assay Kit (K627)
PicoProbe™ Glucose-6-Phosphate Assay Kit (K687)
NAD/NADH Quantification Kit (K337)
ATP Colorimetric Assay Kit (K354)
ADP Colorimetric Assay Kit (K356)

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