Elite™ Glucose Colorimetric/Fluorometric Assay Kit

CATALOG NUMBER: CA-G005, 500 assays (5x 96-well plate)

Description

Glucose ($C_6H_{12}O_6$) is a ubiquitous fuel in biology. It is used as an energy source in most organisms, from bacteria to humans. Glucose level is a key diagnostic parameter for many metabolic disorders, such as diabetes.

The Elite™ Glucose assay kit provides a quick and sensitive method for the measurement of glucose. It uses glucose oxidase-based enzyme coupled reactions to detect glucose through the production of hydrogen peroxide, which is monitored by our Elite™ peroxidase substrate. Elite™ peroxidase substrate can be recorded in a dual mode: colorimetric assay at 570 nm and fluorometric assay at Ex/Em = 540/590 nm. The assay is robust, and can be readily adapted for a wide variety of applications that require the measurement of glucose. The assay has very low background since it is run in the red visible range that significantly reduces the interference from biological samples. It has demonstrated high sensitivity and low interference with excitation at 570 nm and emission at 590 nm. When using fluorometric detection, we can detect as little as 3 µM D-glucose.

Kit Components

• **Component A**: Elite™ Peroxidase Substrate (light sensitive)	1 vial
• **Component B**: Assay Buffer	1 bottle (50 ml)
• **Component C**: Horseradish Peroxidase (HRP)	1 vial (10 units)
• **Component D**: Glucose Oxidase	1 vial (100 units)
• **Component E**: DMSO	1 vial (200 µl)
• **Component F**: Glucose Standard	1 vial (144 mg)

Storage

Keep **Component A** at -20 ºC and avoid exposure to light; **Component C&D** at -20 ºC; **Component B, E&F** at 4 ºC. All components are stable for 6 months after receipt if stored properly.

Shelf Life

All reagents are stable for 6 month after receipt when stored properly at the recommended conditions.

Materials Required (but not supplied)

- 96 microplates: Tissue culture microplate with black wall and clear bottom is recommended.
- Fluorescence microplate reader.

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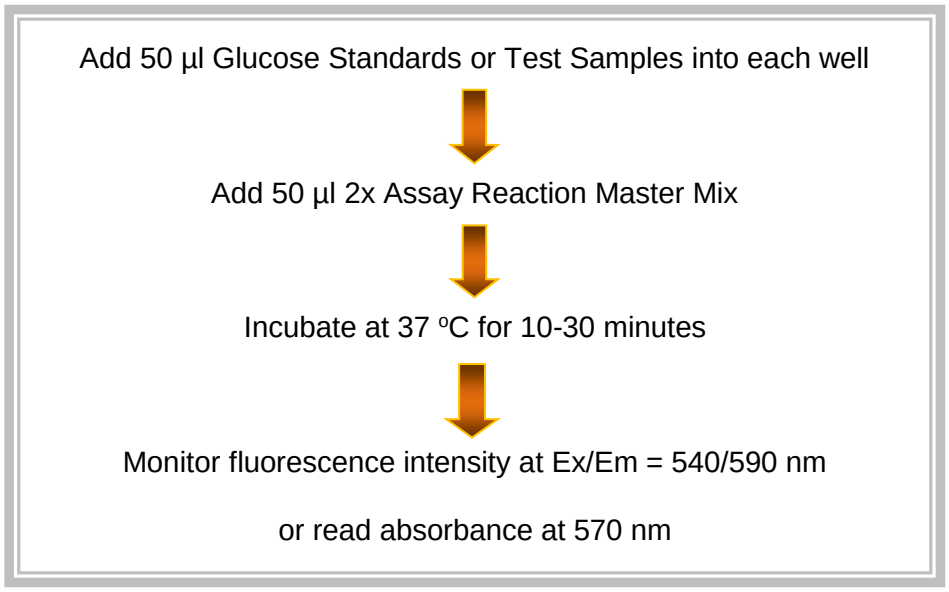
Assay Protocol

Summary of the Assay



1. Preparation of Stock Solutions:

1.1 250x Elite™ Peroxidase Substrate Stock Solution: Add 100 µl of DMSO (**Component E**) into the vial of Elite™ Peroxidase Substrate (**Component A**) to make 250x Elite™ Peroxidase Substrate stock solution.

Note: *The unused 250x Elite™ Peroxidase Substrate stock solution should be divided into single use aliquots and stored at -20 °C.*

Note: *The Elite™ Peroxidase Substrate is unstable in the presence of thiols such as dithiothreitol (DTT) and 2-mercaptoethanol. The final concentration of DTT or 2-mercaptoethanol in the reaction should be no higher than 10 µM. The Elite™ Peroxidase Substrate is also unstable at high pH (>8.5). Therefore, the reaction should be performed at pH7-8. The provided assay buffer (pH 7.4) is recommended.*

1.2 HRP Stock Solution (10 U/ml): add 1 ml of assay buffer (**Component B**) into the vial of horseradish peroxidase (**Component C**).

Note: *the unused HRP solution should be divided into small aliquots and kept at -20 °C.*

1.3 Glucose Oxidase Solution (100 U/ml): add 1 ml of assay buffer (**Component B**) into the vial of glucose oxidase (**Component D**).

Note: *the unused glucose oxidase solution should be divided into small aliquots and kept at -20 °C.*

1.4 Glucose Standard Stock Solution (800 mM): add 1 ml of assay buffer (**Component B**) into the vial of glucose standard (**Component F**).

Note: *the unused glucose standard solution should be kept at -20 °C.*

2. **Preparation of Assay Reaction Master Mix:**

Prepare Assay Reaction Master Mix (2x) according to the Table 1 (protected from light).

Table 1. Assay Reaction Master Mix (2x) for one 96-well plate

Components	Volume
250x Elite™ Peroxidase Substrate Stock Solution (from Step 1.1)	20 µl

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HRP Stock Solution (10 U/ml) (from Step 1.2)	100 µl
Glucose Oxidase Solution (100 U/ml) (from Step 1.3)	100 µl
Assay Buffer (**Component B**)	4.78 ml
Total volume	**5 ml**

3. Preparation of Serial Dilutions of Glucose Standard (0 to 100 µM):

3.1. Prepare a glucose standard by diluting the appropriate amount of the 800 mM Glucose Stock Solution (from Step 1.4) into assay buffer (**Component B**) to produce glucose concentrations of 0 to 200 µM, each in a volume of 50 µl. A non-glucose buffer control is included as blank control. The final glucose concentrations should be twofold lower (i.e., 0 to 100 µM).

3.2. Add serially diluted glucose standards and glucose-containing test samples into a solid black 96-well microplate as described in Tables 2.

Table 2. Layout of glucose standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS								
GS1	GS1										
GS2	GS2										
GS3	GS3										
GS4	GS4										
GS5	GS5										
GS6	GS6										
GS7	GS7										

Note: GS= Glucose Standards; BL=Blank Control; TS=Test Samples.

Table 3. Reagent composition for each well

Glucose Standards	**Blank Control**	**Test Sample**
Serial Dilutions: 50 µl	Assay Buffer: 50 µl	50 µl

Note 1: *Add the serially diluted glucose standards from 3 µM to 200 µM into wells from GS1 to GS7 in duplicate.*

Note 2: *High concentration of glucose (e.g., 500 µM, final concentration) may cause reduced fluorescence signal due to the overoxidation of Elite™ Peroxidase Substrate (to a non-fluorescence product).*

4. Run Glucose Assay:

4.1. Add 50 µl of 2x Assay Reaction Master Mix (from Step 2) into each well of glucose standard, blank control, and test samples (see Table 2) to make the total glucose assay volume of 100 µl/well.

Note: *For a 384-well plate, add 25 µl of test sample (**see Appendix A**) and 25 µl of 2x Assay Reaction Master Mix into each well.*

4.2. Incubate the reaction for 10 to 30 minutes at 37 °C (protected from light).

4.3. Monitor the fluorescence intensity at Ex/Em = 530-570 nm/490-600 nm using a fluorescence plate reader (optimal Ex/Em = 540/590 nm).

Note: *The contents of the plate can also be transferred to a white clear bottom plate and read by an absorbance microplate reader at the wavelength of 576 ± 5 nm. The absorption detection has lower sensitivity compared to fluorescence reading.*

Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and subtracted from the values of those wells with the glucose reactions. A glucose standard curve is shown in Figure 1.

Note: *The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.*

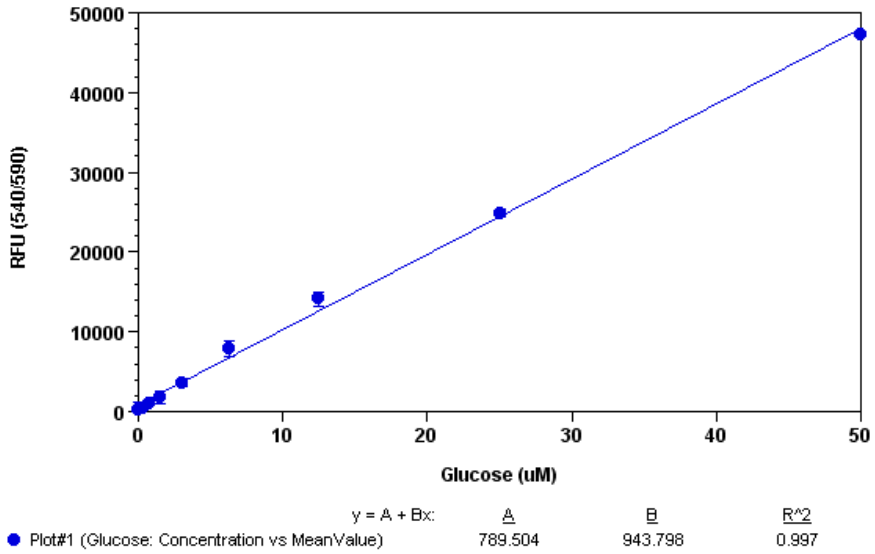

Figure 1. Glucose dose response was measured with Elite™ Glucose Colorimetric/Flurorimetric Assay Kit on a 96-well black plate using a Novostar microplate reader. As low as 3 µM glucose was detected with 30 minutes' incubation (n = 3).

Appendix A

Sample lysis preparation for the assay kits

This protocol serves as a general guide for preparing samples for the assays. For the best results, users need to adjust the amount of materials for their unique cell samples.

1. Lysis of plant cells

   Homogenize the leaves with the lysis buffer (Cat# CA-N015g) at 200 mg/ml, and centrifuge at 2500 rpm for 5-1o minutes. Use the supernatant for the assay.

2. Lysis of bacterial cells

   Collect bacterial cells by centrifugation (i.e. 10,000 x g, 0°C, 15min). Add lysis buffer (Cat# CA-N015g) to the pellet (1 mL per 100 to 10,000,000 cells), and leave at room temperature for 15 minutes. Centrifuge at 2500 rpm for 5 minutes, and use the supernatant for the assay.

3. Lysis of mammalian cells

   Remove the medium from the culture plate (wells). Use about 100 µL lysis buffer (Cat# CA-N015g) per 1-5 million cells (or add lysis buffer 100 µL lysis buffer per well in a 96-well cell culture plate), and leave at room temperature for 15 minutes. For the assay, use the lysate directly or centrifuge at 1500 rpm for 5min then use the supernatant.

4. Lysis of tissues

   Weigh ~ 20 mg tissue, then wash with cold PBS buffer. Homogenize the tissue with 400 µl of lysis buffer (Cat# CA-N015g) in a micro-centrifuge tube, and centrifuge at 2500 rpm for 5-10 minutes. Use the supernatant for the assay.