

## **Technical Manual**

## **ColorFluor Glucose Assay Kit**

**Catalogue Code: BA0081**

**Pack Size: 100 assays**

**Research Use Only**

## DESCRIPTION

Glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>) is a key diagnostic parameter for many metabolic disorders. Increased glucose levels have been associated with diabetes mellitus, hyperactivity of thyroid, pituitary and adrenal glands. Decreased levels are found in insulin secreting tumors, myxedema, hypopituitarism and hypoadrenalism. Simple, direct and high-throughput assays for measuring glucose concentrations find wide applications in research and drug discovery. The Assay Genie ColorFluor Glucose Assay Kit uses a single Working Reagent that combines the glucose oxidase reaction and color reaction in one step. The color intensity of the reaction product at 570nm or fluorescence intensity at  $\lambda_{em/ex} = 585/530nm$  is directly proportional to glucose concentration in the sample.

## KEY FEATURES

**Sensitive and accurate.** Use as little as 20  $\mu$ L samples. Linear detection range in 96-well plate: 5 to 300  $\mu$ M (90  $\mu$ g/dL to 5.4 mg/dL) glucose for colorimetric assays and 1 to 30  $\mu$ M for fluorimetric assays.

**Simple and high-throughput.** The procedure involves addition of a single working reagent and incubation for 30 min at room temperature.

## APPLICATIONS:

**Direct Assays:** glucose in serum, plasma, urine, saliva, milk, culture medium and other biological samples.

**Drug Discovery/Pharmacology:** effects of drugs on glucose metabolism.

**Food and Beverages:** glucose in food, beverages etc.

## KIT CONTENTS:

**Assay Buffer:** 10 mL    **Enzyme Mix:** 120  $\mu$ L

**Dye Reagent:** 120  $\mu$ L    **Standard:** 1 mL 300 mg/dL Glucose

**Storage conditions.** The kit is shipped on ice. Store Assay Buffer at 4°C and other components at -20°C. Shelf life: 6 months after receipt.

**Precautions:** reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

## COLORIMETRIC PROCEDURE

**Sample treatment:** saliva samples should be centrifuged for 5 min at 14,000 rpm prior to assay. Milk samples should be cleared by mixing 100  $\mu$ L 6N HCl and 600  $\mu$ L milk. Centrifuge 5 min at 14,000 rpm and transfer supernatant into a clean tube. Add 170  $\mu$ L 6N NaOH per mL supernatant. Mix well and centrifuge again at 14,000 rpm for 5 min. The supernatant can be assayed. The dilution factor in this procedure is  $n = 1.36$ .

Samples can be analyzed immediately after collection, or stored in aliquots at -20 °C. Avoid repeated freeze-thaw cycles. If particulates are present, centrifuge sample and use clear supernatant for assay.

1. Equilibrate all components to room temperature. During experiment, keep thawed Enzyme in a refrigerator or on ice.
2. *Standards and samples:* for 300  $\mu$ M standard, mix 15  $\mu$ L 300 mg/dL standard with 818  $\mu$ L dH<sub>2</sub>O. Dilute standard in dH<sub>2</sub>O as follows.

No	300 $\mu$ M STD + H <sub>2</sub> O	Vol ( $\mu$ L)	Glucose ( $\mu$ M)
1	200 $\mu$ L + 0 $\mu$ L	200	300
2	120 $\mu$ L + 80 $\mu$ L	200	180
3	60 $\mu$ L + 140 $\mu$ L	200	90
4	0 $\mu$ L + 200 $\mu$ L	200	0

Transfer 20  $\mu\text{L}$  standards and samples into separate wells.

3. *Working Reagent*. For each reaction well, mix 85  $\mu\text{L}$  Assay Buffer, 1  $\mu\text{L}$  Enzyme Mix (*vortex briefly before pipetting*), and 1  $\mu\text{L}$  Dye Reagent in a clean tube. Transfer 80  $\mu\text{L}$  Working Reagent into each reaction well. Tap plate to mix.

4. Incubate 30 min at room temperature. Read optical density at 570nm (550-585nm).

### CALCULATION

Subtract blank OD (water, #4) from the standard OD values and plot the  $\Delta\text{OD}$  against standard concentrations. Determine the slope and calculate the glucose concentration of Sample,

$$[\text{Glucose}] = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{Slope}} \quad (\mu\text{M})$$

$\text{OD}_{\text{SAMPLE}}$ ,  $\text{OD}_{\text{BLANK}}$  are optical density values of the sample and water.

**Conversions:** 1 mg/dL glucose equals 55.5  $\mu\text{M}$ , 0.001% or 10 ppm.

### FLUORIMETRIC PROCEDURE

For fluorimetric assays, the linear detection range is 1 to 30  $\mu\text{M}$  glucose. Mix 20  $\mu\text{L}$  of the standards from *Colorimetric Procedure* with 180  $\mu\text{L}$   $\text{dH}_2\text{O}$  to obtain standards at 30, 18, 9, 0  $\mu\text{M}$  glucose.

Transfer 20  $\mu\text{L}$  standards and 20  $\mu\text{L}$  samples into separate wells of a *black* 96-well plate.

Add 80  $\mu\text{L}$  Working Reagent (see *Colorimetric Procedure*), tap plate to mix.

Incubate 30 min at room temperature and read fluorescence at  $\lambda_{\text{ex}} = 530\text{nm}$  and  $\lambda_{\text{em}} = 585\text{nm}$ .

The glucose concentration of Sample is calculated as

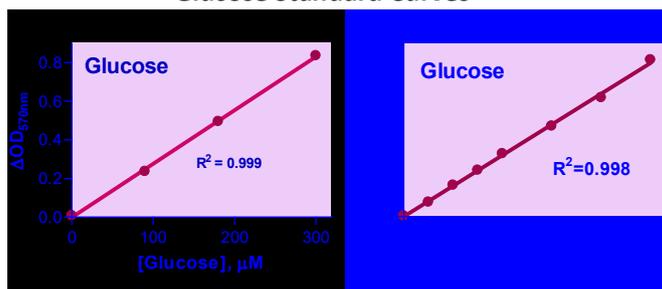
$$[\text{Glucose}] = \frac{F_{\text{SAMPLE}} - F_{\text{BLANK}}}{\text{Slope}} \quad (\mu\text{M})$$

**Notes:** (1). If the calculated sample glucose concentration is higher than 300  $\mu\text{M}$  in colorimetric assay or 30  $\mu\text{M}$  in fluorimetric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor. (2). To determine glucose in phenol red culture medium, dilute both sample and glucose standards in the same glucose free medium for colorimetric assay. For fluorimetric assay, prepare standards in phenol red medium. Dilute sample and standards 20-fold or more in water.

### MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates, black 96-well plates and plate reader.

**Glucose Standard Curves**



*96-well colorimetric assay    96-well fluorimetric assay*

**LITERATURE**

1. Okuda J, Okuda G. (1969). A rapid polarographic microdeter-mination of glucose with glucose oxidase. Clin Chim Acta. 23(2):365-7.
2. Saifer A, Gerstenfeld S. (1958). The photometric microdeter-mination of blood glucose with glucose oxidase. J Lab Clin Med. 51(3):448-60.
3. Middleton JE, Griffiths WJ. (1957). Rapid colorimetric micro-method for estimating glucose in blood and C. S. F. using glucose oxidase. Br Med J. 2(5060):1525-7.

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