A convenient, easy-to-follow shortened protocol is now being provided with this assay.
For a detailed protocol go to www.caymanchem.com/pdfs/10009582.pdf

Glucose Colorimetric Assay Kit Short Protocol

Item No. 10009582

REAGENT PREPARATION

1. **Glucose Assay Standard - (1,000 mg/dL; Item No. 10010098)** - This standard is ready to use as supplied.

2. **Sodium Phosphate Assay Buffer - (Item No. 700003)** - Dilute the contents with 40 ml of HPLC-grade water (final formulation is 50 mM sodium phosphate, pH 7.2); stable for three months at 4°C.

3. **Glucose Colorimetric Enzyme Mixture - (Item No. 10010100)** - Reconstitute vial (1 vial sufficient for 60 wells) with 6 ml of diluted Assay Buffer and mix well; stable for one hour at 4°C.

STANDARD PREPARATION

Dilute 50 µl of the 1,000 mg/dL Glucose Standard with 450 µl of diluted Assay Buffer to make a 100 mg/dL stock. Prepare Assay Standards as shown in Table 1. Standards are stable for 2 hrs at room temperature.

Tube	Glucose Stock (µl) (100 mg/dL)	Assay Buffer (µl)	Glucose Concentration (mg/dL)
A	0	200	0
B	5	195	2.5
C	10	190	5
D	15	185	7.5
E	20	180	10
F	30	170	15
G	40	160	20
H	50	150	25

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PERFORMING THE ASSAY

1. **Glucose Standard wells** - Add 85 µl of diluted Assay Buffer and 15 µl of each Standard (tubes A-H) to duplicate wells.
2. **Sample wells** - Add 85 µl of diluted Assay Buffer and 15 µl of sample to duplicate wells.
3. **Initiate the reaction** - Add 100 µl of Enzyme Mixture to all standard and sample wells.
4. **Cover** plate and **Incubate** for 10 minutes at 37°C.
5. **Remove** the plate cover and **read** the absorbance at 500-520 nm.

CALCULATIONS

1. Calculate the average absorbance of each standard and sample.
2. Subtract the absorbance value of the standard A (0 mg/dL) from itself and all other values (both standards and samples). This is the corrected absorbance.
3. Plot the corrected absorbance values of each standard as a function of the concentration of glucose and fit the data to a linear regression.
4. Calculate the concentration of glucose for each sample from the standard curve using the equation below.

$$\text{Glucose (mg/dL)} = \left[\frac{\text{(Corrected absorbance) - (y-intercept)}}{\text{Slope}}\right] \text{ x dilution}$$