BIOMEDICAL RESEARCH SERVICE CENTER
UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

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Glucose Assay kit (Cat #: A-114)

COMPONENTS: Glucose Assay Solution: 10 ml; store in aliquots at -80℃ (for 200 assays)
1 M Glucose Standard: 0.1 ml; store at 4℃

PRODUCT DESCRIPTION: In humans, glucose homeostasis under different metabolic circumstances is of vital importance, and insulin regulates the concentration of glucose in the blood such that its concentration normally falls within 3.5 − 8 mM. Abnormal circulating levels of glucose are associated with major pathologic states such as diabetes and obesity. The Glucose Assay Kit is based on sequential hexokinase and glucose-6-phosphate dehydrogenase reactions in a $NADP^+$-coupled oxidation-reduction. The Glucose Assay kit is used to measure the concentration of glucose with a linear detection range of 0.1 – 1 mM. The assay solution is stable for many years if stored in aliquots shielded from light at -80℃.

PROTOCOL:

***Assay solution and glucose standard:** Quickly thaw Glucose Assay Solution and keep solution on ice during assay. Freeze the assay solution in small aliquots after the first use. Dilute the 1 M glucose standard 1,000-fold with dH_2O to obtain a 1 mM glucose standard, e.g., 1 µl of 1M glucose + 1 ml dH_2O. Perform serial dilutions to obtain 0.5, 0.25, and 0.125 mM glucose standards.

****Samples containing plasma or serum:** Thawed serum/plasma or culture medium samples should be kept on ice. Culture medium and plasma or serum (preferred) samples, which typically contain glucose at mM levels, need to be diluted 10-fold with dH_2O for the assay. Note that all samples should be equally diluted. The final result needs to be multiplied by the dilution factor.

*****Cell and tissue samples:** Cell and tissue samples can be homogenized in TCA solution. Please follow the protocol at http://www.bmrservice.com/SupplementTCA.html. Plasma/serum/medium samples can also be extracted by the TCA method for glucose assay.

******Urine samples:** Add 1 ml urine to a 1.5-ml microtube and seal tube well. Place tube in a boiling water bath for 10 min, following which the boiled urine is clarified by centrifugation at maximum speed for 5 min. Transfer supernatant to another microtube and store at -80℃.

ASSAY:

1. Add 10 µl glucose standards and samples to a 96-well microplate. Add 10 µl dH_2O to a well as blank. Enzymatic reaction is initiated by addition of 50 µl Glucose Assay Solution to each well. Add solution swiftly to minimize assay variation.

2. Quickly mix contents by gentle but thorough agitation. Cover plate and incubate at 37°C 30 min.

3. Stop reaction by adding 50 µl 3% (or 0.5 M) acetic acid per well followed by brief gentle agitation. Eliminate air bubbles present in the wells prior to measurement. Measure absorbance at 492 nm using a microplate reader. The blank reading should be subtracted from all sample readings.

4. Generate a glucose standard graph first. Plot the data to obtain glucose concentrations of the samples. A new plot must be generated for each assay. Multiply result by the dilution factor where applicable.

ADDITIONAL INFORMATION:

- Glucose Assay Solution contains the organic solvent DMSO (9% v/v) and iodonitrotetrazolium violet (2 mg/ml). Please contact us or visit the product webpage for MSDS information.

- A 3% acetic acid solution needs to be prepared for reaction termination.