BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Glucose Assay kit (Cat #: A-114)

COMPONENTS: Glucose Assay Solution: 5 ml (for 100 assays), store at -80°C **(shield from light during use)**
20 mM Glucose: 0.5 ml, store at -20°C or -80°C
PEG Solution: 3 ml; store at room temperature **(agitate vial to redissolve contents upon arrival)**

PRODUCT DESCRIPTION: The Glucose Assay Kit is based on sequential hexokinase and glucose-6-phosphate dehydrogenase reactions in a $NADP^+$-coupled oxidation-reduction. The assay is used to measure the concentration of glucose present in biological fluids and cell culture medium with a linear detection range of 10 – 1000 µM. The assay solution is stable for several years if stored and handled properly.

PROTOCOLS

Preparation of serum/plasma and cell culture medium: These samples are deproteinized by PEG precipitation. Mix 25 µl of each sample with 25 µl PEG Solution in a 1.5-ml microtube (PEG solution should be pipetted slowly using a cut tip). Shake tube vigorously followed by vortexing to ensure thorough mixing. Keep tube on ice for 3 min. Centrifuge solution in a microfuge at ~14,000 rpm for 3 min. Harvest supernatant and store at -20°C.

Preparation of tissue/cell samples: Cell and tissue samples are deproteinized by PEG precipitation. Please follow the extraction protocol at https://bmrservice.com/deproteination-by-peg. Alternatively, the samples can be deproteinized by TCA precipitation (https://bmrservice.com/deproteination-by-tca). Deproteinized samples may be diluted with dH_2O prior to assay to achieve optimal results.

Glucose standard: Dilute the 20 mM glucose standard 100-fold with dH_2O to 200 µM, e.g., 495 µl dH_2O + 5 µl 20 mM glucose. Perform 1:1 serial dilution to generate 100 µM, 50 µM, and 25 µM glucose standards. Store diluted standards at -20°C.

ASSAY

1. Serum and cell culture medium samples usually contain glucose at mM levels and should be properly diluted with ice-cold dH_2O after deproteinization prior to assay to ensure assay linearity.
2. Thaw Glucose Assay Solution and keep solution on ice and shielded from light. *Do not over thaw solution.*
3. Add 20 µl of each glucose standard and properly diluted sample to a plain (uncoated) 96-well microplate.
4. Gently agitate Glucose Assay Solution prior to first pipetting. Assay is initiated by addition of 50 µl Glucose Assay Solution to each well. Mix contents by gentle agitation. Cover plate and incubate at 37°C for 30 min. *Do not use CO_2 incubator.*
5. Eliminate air bubbles present in wells prior to measurement if necessary. Measure absorbance at 492 nm using a plate reader.
6. Plot glucose standards vs. $O.D._{492\ nm}$. Generate a trend line equation on chart (see graph below).
7. Calculate sample glucose concentration using the derived equation (x = glucose concentration in µM; y = $O.D._{492\ nm}$). A new plot must be generated for each assay. Multiply sample glucose concentration by the applicable dilution factor.

ADDITIONAL INFORMATION:

- Glucose Assay Solution contains the organic solvent dimethyl sulfoxide (DMSO) and iodonitrotetrazolium chloride. Please contact us or visit the product webpage for material SDS information.
- Agitate the PEG Solution vial in warm water to re-dissolve contents upon arrival.