BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO
Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 955 Main St., Suite 4102, Buffalo, NY 14203
Tel: (716) 4586903 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Hexokinase/Glucokinase (HK/GK) Assay Kit (Cat #: E-111)

COMPONENTS: HK Assay Solution- 5 ml (100 wells), store at -80ºC **(shield solution from light during assay)**
HK Substrate Solution (50x)- 0.5 ml, store at 4ºC
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents)**

PRODUCT DESCRIPTION: The HK/GK assay is based on the reduction of the tetrazolium salt INT in a NADPH-coupled reaction to formazan, which exhibits an absorption maximum at 492 nm (extinction coefficient = 18 $mM^{-1}cm^{-1}$) and allows for measurement of HK in tissue/cell lysates. Kit components are stable for several years if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Bring up ~10^5 washed cells in 50–100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~5 mg tissue in 0.5 ml).

2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.

3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 0.2 – 2 mg/ml.

Reagent thawing:

Keep thawed HK Assay Solution on ice and shielded from light. *Do not over thaw*. Freeze solution immediately after use.

Preparation of control solution and reaction solution:

Control solution is prepared by mixing 1 part of dH_2O and 50 parts of HK Assay Solution, e.g. 10 µl dH_2O mixed with 500 µl HK Assay Solution.

Reaction solution is prepared by mixing 1 part of HK Substrate Solution and 50 parts of HK Assay Solution, e.g. 10 µl HK Substrate Solution mixed with 500 µl HK Assay Solution.

Enzyme assay:

1. Add 10 µl of each sample to duplicate wells of a plain (uncoated) 96-well plate (for control set and reaction set)

2. Add 50 µl control solution to one set of wells (control wells) and 50 µl reaction solution to the other set of wells (reaction wells). Mix contents by gentle agitation for 10 sec.

3. Cover plate and incubate in a 37°C incubator for 60 min or 90 min. *Do not use CO_2 incubator*. Cherry red color should gradually appear in sample wells.

3. Measure $O.D._{492\ nm}$ using a plate reader. Subtract control well reading from reaction well reading to generate **ΔO.D.** for each sample.

4. If incubation for 60 min, sample HK enzyme activity in IU/L = µmol/(L•min) = **ΔO.D.** × 1000 × 60 µl/(60 min × 0.4 cm × 18 × 10 µl) = **ΔO.D. × 13.89**. If incubation for 90 min, HK activity = **ΔO.D. x 9.26**. Enzyme activity may be presented as units/µg proteins or normalized by GAPDH enzyme activity (see cat#E-101).

Additional information:

- The HK Assay Solution contains dimethyl sulfoxide (DMSO) and iodonitrotetrazolium (INT). Please refer to the product webpage or contact us for material SDS information.
- 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.