

BIOMEDICAL RESEARCH SERVICE CENTER

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

COMPONENTS: HK Assay Solution- 10 ml (for 200 wells); **store in aliquots at -80°C after first thawing**
HK Substrate (50x)- 0.5 ml, store at 4°C
10x Cell Lysis Solution- 25 ml, store at 4°C

PRODUCT DESCRIPTION: The HK assay is based on the reduction of the tetrazolium salt INT in a NADH-coupled reaction to INT-formazan, which exhibits an absorption maximum at 492 nm (molar extinction coefficient = $18 \text{ mM}^{-1}\text{cm}^{-1}$) and allows for sensitive detection of HK in serum and tissue samples. Assay solution is stable for several years if handled properly.

Plasma: Plasma samples may need to be diluted with ice-cold 1x Cell Lysis Solution (from 10-fold dilution of 10x Cell Lysis Solution with dH₂O) to obtain assay linearity. Samples should be store at -80°C.

Preparation of cell/tissue extracts:

1. Wash $10^5 - 10^6$ cells with ice-cold phosphate-buffered saline (PBS). Animal tissue should be washed with PBS thoroughly to remove blood cells. Freeze cell pellet and tissue at -80°C until use.
2. Add 50 – 100 μl ice-cold 1x Cell Lysis Solution to cell pellet. Extract cells by pipetting up and down (gently but thoroughly). Leave lysate on ice for 5 min with intermittent gentle agitation. Centrifuge lysate in a refrigerated microfuge for 3 min at maximum speed ($\sim 13,000$ rpm) and harvest supernatant for HK assay. For tissue extraction, weigh ~ 50 mg tissue and homogenize in 1 ml ice-cold 1x Cell Lysis Solution. Centrifuge homogenate at 4°C for 3 min at maximum speed and harvest supernatant. Store cell lysate and tissue homogenate at -80°C.
3. Perform protein assay to determine sample protein concentration. Equalize sample protein concentration by diluting with ice-cold 1x Cell Lysis Solution. A suggested protein concentration range is 0.2 – 1 mg/ml.

Reagent thawing:

Keep thawed HK Assay Solution on ice. Gently agitate solution prior to pipetting. It is important to minimize the time the solution is thawed. Freeze solution immediately after use.

Preparation of control solution and reaction solution:

Control solution is prepared by mixing 1 part of dH₂O and 50 parts of HK Assay Solution, e.g. 10 μl dH₂O mixed with 500 μl HK Assay Solution. Keep freshly prepared control solution on ice during assay.

Reaction solution is prepared by mixing 1 part of HK substrate and 50 parts of HK Assay Solution, e.g. 10 μl HK Substrate mixed with 500 μl HK Assay Solution. Keep freshly prepared reaction solution on ice during assay.

Estimate the volume of control solution and reaction solution required for each assay. Each sample is treated with 50 μl control solution and 50 μl reaction solution in separate wells (see below).

Enzyme assay:

1. Add 10 μl of each sample to a 96-well plate in duplicate: one set for control and another for reaction.
2. After all samples have been pipetted to the plate in duplicate, add 50 μl control solution to one set of wells and 50 μl reaction solution to another set of wells. Gently agitate plate for 30 sec. Cover plate and incubate in a humidified 37°C incubator for 30 min or 60 min (for low activity). Do not use CO₂ incubator.
3. Stop assay by adding 50 μl 3% Acetic acid (not included in the kit) to each control solution well and reaction solution well followed by brief gentle agitation. Measure O.D._{492 nm} using a plate reader. Subtract control well reading from reaction well reading for each sample. Use the subtracted sample reading (**O.D.**) for enzyme activity calculation shown below.
4. For 30 min reaction, sample HK activity in IU/L unit = $\mu\text{mol}/(\text{L}\cdot\text{min}) = \text{O.D.} \times 1000 \times 110 \mu\text{l} / (30 \text{ min} \times 0.6 \text{ cm} \times 18 \times 10 \mu\text{l}) = \text{O.D.} \times 33.96$. For 60 min, HK activity in IU/L unit = **O.D. \times 16.98**. Note that sample dilution may be desired to achieve assay linearity. Multiply the result by the dilution factor where applicable.

Additional information:

- A 3% Acetic acid solution needs to be prepared for reaction termination.
- The “0.6 cm” in the equation is the typical light path in a 96-well plate and may be custom adjusted as needed.
- The HK Assay Solution contains DMSO and iodonitrotetrazolium violet. Please refer to the product page of our website or contact us for MSDS information.