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Instructions for Use

Version: 1.0.1
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Hexokinase Assay Kit

Catalog No.: abx298839

Size: 96 tests

Detection Range: 5.93 U/L - 40.11 U/L

Sensitivity: 5.93 U/L

Storage: Store all components in the dark at -20°C.

Application: For detection and quantification of Hexokinase activity in tissue and cell lysates.

Introduction

Hexokinase catalyzes the phosphorylation of hexose to produce hexose phosphate which is important in glucose decomposition. Hexokinase also catalyzes the conversion of glucose to glucose 6-phosphate in carbohydrate metabolism. NAD^+ is reduced to NADH and transfers electrons to WST-8 producing a yellow product because of hydrogen transmission. The activity of hexokinase can be calculated by measuring the change of absorbance value at 450nm.

Abbexa's Hexokinase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Hexokinase activity. The absorbance should be measured at 450 nm. The intensity of the color is proportional to the activity of the Hexokinase enzyme(s), which can then be calculated.

Kit components

1. 96-well microplate
2. Buffer Solution: 25 ml
3. Substrate: 1 vial
4. Enzyme Reagent: 1 vial
5. Detection Reagent: 3 ml
6. Standard (0.8 mmol/L): 3.2 ml
7. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (450 nm)
2. Normal saline (0.9% NaCl)
3. Distilled water
4. Ice water bath
5. Pipette and pipette tips
6. Vials/tubes
7. Incubator
8. Centrifuge

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Protocol

A. Preparation of samples and reagents

1. Reagents

Bring all reagents to room temperature before use.

- **Substrate Working Solution:** Dissolve a vial of Substrate with 12 ml of Buffer Solution and mix to ensure that the solid powder is completely dissolved. The prepared solution can be stored in the dark at -20°C for up to 2 weeks.
- **Enzyme Reagent Working Solution:** Dissolve a vial of Enzyme Reagent with 12 ml of Buffer Solution and mix to ensure that the solid powder is completely dissolved. The prepared solution can be stored in the dark at -20°C for up to 2 weeks.
- **Enzyme Working Solution:** Mix the Substrate Working Solution with the Enzyme Reagent Working Solution at a 1:1 ratio. Prepare the needed amount just before use to ensure it is fresh. The prepared solution cab be stored in the dark at 2-8°C for up to 12 hours.
- **Sample Reaction Solution:** Mix the Enzyme Working Solution and Detection Reagent at a 10:1 ratio. Prepare the needed amount just before use to ensure it is fresh. The prepared solution can be stored in the dark at 2-8°C for up to12 hours.
- **Control Solution:** Mix distilled water and Detection Reagent at a 10:1 ratio. Prepare the needed amount just before use to ensure it is fresh. The prepared solution can be stored in the dark at 2-8°C for up to 12 hours.

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Sample:** Weigh the tissue homogenate. For each 1 g of homogenate, add 9 ml of normal saline (0.9% NaCl). Homogenize by hand, using a mechanical homogenizer, or by ultrasonication in an ice water bath. Centrifuge the homogenate at 10,000 x g at 4°C for 15 min. Collect the supernatant and assay immediately. If a precipitate appears, centrifuge again. The protein concentration in the supernatant should be determined separately.
- **Cell lysates:** Collect cells into a centrifuge tube and wash with PBS 1-2 times. Centrifuge at 1000 × g for 10 min and discard the supernatant. For every $5x10^6$ cells, add 200 µl of Normal Saline (0.9% NaCl), then sonicate in an ice water bath. Centrifuge at 10,000 × g at 4°C for 10 min. Take the supernatant into a new centrifuge tube (while kept on ice) and analyze immediately. The protein concentration in the supernatant should be determined separately.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

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It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

The recommended dilution factor for different samples is as follow (for reference only):

Sample Type	Dilution Factor
10% Mouse Spleen Tissue Homogenate	1
10% Mouse Liver Tissue Homogenate	1
10% Mouse Kidney Tissue Homogenate	1
10% Mouse Heart Tissue Homogenate	2-6
10% Mouse Brain Tissue Homogenate	1
10% Rat Spleen Tissue Homogenate	1
10% Rat Lung Tissue Homogenate	1
10% Rat Liver Tissue Homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.
- The sample diluent is normal saline (0.9% NaCl).

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B. Assay Procedure

1. Set standard, sample and sample control wells on the 96 well microplate and label accordingly. Each sample requires a sample control well.
2. **Standard curve preparation:** Label 8 tubes with 0.8, 0.64, 0.56, 0.48, 0.4, 0.32, 0.16 and 0 mmol/L. Dilute the 0.8 mmol/L standard solution with distilled water to create serial concentrations 0.8, 0.64, 0.56, 0.48, 0.4, 0.32, 0.16 and 0 mmol/L. See the table for serial dilution reference.

Tube	Standard concentration (mmol/L)	0.8 mmol/L standard solution (μl)	Distilled water (μl)
A	0	0	200
B	0.16	40	160
C	0.32	80	120
D	0.4	100	100
E	0.48	120	80
F	0.56	140	60
G	0.64	160	40
H	0.8	200	0

3. Add 10 μl of each standard solution to the standard wells.
4. Add 10 μl of sample to the control wells .
5. Add 10 μl of sample to the sample wells.
6. Add 220 μl of Sample Reaction Solution to the standard wells and sample wells.
7. Add 220 μl of Control Solution to the control wells.
8. Incubate at 37°C for 10 minutes.
9. Measure the OD value of each well at 450nm with a microplate reader

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C. Calculation of Results

The standard curve can be plotted as the absolute OD_{540} of each standard solution (*y*) vs. the respective concentration of the standard solution (*x*). A linear fit is recommended for the standard curve (*y* = *ax* + b). Create the standard curve with graph software. The Hexokinase concentration of the samples can be interpolated from the standard curve.

One unit is defined at the amount of Hexokinase in 1 g of tissue or cell protein per 1 minute of hydrolyzing the substrate to produce 1 μmol of gluocose-6-phosphate at 37°C

$$\text{Hexokinase activity (U/gprot)} = \frac{\Delta A_{450} - b}{a \times T} \times \frac{1000}{C_{\text{Pr}}} \times f$$

where:

y	$OD_{Standard}$ - OD_{Blank}
x	concentration of Standard
a	gradient of the standard curve
b	y-intercept of the standard curve
ΔA_{540}	OD_{Sample} – $OD_{Control}$
T	time of the reaction (10 minutes)
C_{Pr}	concentration of protein in sample (gprot/L)
f	dilution factor of the sample before carrying out the assay
1000	1 mmol = 1000 μmol

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