Product Manual

Hexokinase (HK) Activity Assay Kit

Catalog Number

MET-5087 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Hexokinases (HK) are key enzymes of glucose metabolism, broadly distributed in many species (bacteria, humans, plants). There are four hexokinase isoforms existing in mammals (HK-I, II, III, and IV), each with differences in size, substrates, kinetics, and cellular localization. During glycolysis, hexokinase is responsible for phosphorylating glucose to yield glucose-6-phosphate (step 1 of the pathway). Some tumor cells overexpress specific glycolytic enzymes, such as hexokinase, which allow them to perform glycolysis at much higher rates. This makes hexokinase a potential therapeutic target for several cancers.

Cell Biolabs' Hexokinase Activity Kit measures HK activity in cell and tissue samples. First, hexokinase-containing samples phosphorylate the kit's substrate. Next, this substrate is oxidized, producing a coenzyme product that reacts with the kit's Colorimetric Probe (absorbance maxima of 450 nm).

The HK Activity Assay Kit is a simple, colorimetric assay that quantitatively measures the HK activity in cell/tissue lysates in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, standards, and unknown samples. The kit contains an enzyme standard and has a detection sensitivity limit of <1 mUnits/mL.

Related Products

1. MET-5019: Total Phosphatidic Acid Assay Kit (Fluorometric)
2. MET-5024: Phosphatidylglycerol/Cardiolipin Assay Kit (Fluorometric)
3. MET-5028: DAG (Diacylglycerol) Assay Kit (Fluorometric)
4. MET-5036: DAG Kinase Activity Assay Kit
5. MET-5078: Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Activity Assay Kit
6. MET-5081: Glucose-6-Phosphate Dehydrogenase (G6PDH) Activity Assay Kit
7. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
8. STA-390: Total Cholesterol Assay Kit
9. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
10. STA-394: HDL Cholesterol Assay Kit
11. STA-396: Serum Triglyceride Quantification Kit (Colorimetric)
12. STA-398: Free Glycerol Assay Kit (Colorimetric)
13. STA-618: Free Fatty Acid Assay Kit (Colorimetric)

Kit Components

1. HK Enzyme Standard (Part No. 50871D): One 1 mL vial containing 500 mUnits/mL of hexokinase.

   Note: One unit corresponds to the amount of enzyme which will phosphorylate 1 µmole of D-glucose per minute at pH 7.6 and 25ºC.

2. 5X Lysis Buffer (Part No. 50782B): One 30 mL bottle.
3. Assay Buffer (Part No. 50783B): Two 1.5 mL vials.
4. 10X HK Substrate (Part No. 50872D): One 1 mL vial.
5. 10X Cofactor Mix (Part No. 50873D): One 1 mL vial.
6. 100X Enzyme Mix (Part No. 50874D): One 100 µL vial.
7. Colorimetric Probe (Part No. 50181C): One 1 mL amber vial.

Materials Not Supplied

1. PBS
2. 96-well microtiter plate
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm

Storage

Store the entire kit at -80ºC. Avoid multiple freeze/thaws by aliquoting. The Colorimetric Probe is light sensitive and should be maintained in amber tubes.

Preparation of Reagents

- 1X Lysis Buffer: Dilute the 5X Lysis Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Assay Buffer and Colorimetric Probe: Thaw and maintain at room temperature during assay preparation. Mix well. Any unused material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.
- HK Enzyme Standard, 10X HK Substrate, 10X Cofactor Mix, and 100X Enzyme Mix: Thaw and maintain at 4ºC during assay preparation. Mix well. Any unused material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.

Preparation of HK Enzyme Standard Curve

- HK Enzyme Standard should be thawed/maintained at 4ºC during assay preparation. For longer term storage, the HK Enzyme Standard should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.

- Freshly prepare a dilution series of HK Enzyme Standard in the concentration range of 50 mUnits/mL – 0.78 mUnits/mL by diluting the enzyme stock solution (provided at 500 mUnits/mL) in 1X Lysis Buffer (see Table 1). Standards should be prepared fresh, vortexed well and used immediately. Do not store diluted standards.

Standard Tubes	500 mUnits/mL HK Enzyme Standard (µL)	1X Lysis Buffer (µL)	Final HK Enzyme Standard (mUnits/mL)
1	50	450	50
2	250 of Tube #1	250	25
3	250 of Tube #2	250	12.5
4	250 of Tube #3	250	6.25
5	250 of Tube #4	250	3.13
6	250 of Tube #5	250	1.56
7	250 of Tube #6	250	0.78
8	0	250	0

Table 1. Preparation of HK Enzyme Standard Curve

Preparation of Samples

- Plasma and serum: This kit is not recommended for these samples**.**
- Tissue Samples: Weigh out 100 mg of tissue and mince into small pieces. Rinse the tissue with cold PBS to remove red blood cells and clots. Homogenize the minced tissue in 1 mL cold 1X Lysis Buffer. Centrifuge at 10,000 x g for 10 minutes at 4°C. Carefully collect the supernatant and store on ice for immediate use. For longer term storage, freeze the homogenate at -80°C for up to 1 month. Tissue homogenates may need to be further diluted in cold 1X Lysis Buffer before assaying.
- Suspension Cells: Collect 1 x 10^7 cells by centrifugation at 1000 x g for 10 minutes. Carefully aspirate the culture media and wash once with cold PBS. Centrifuge at 1000 x g for 10 minutes at 4°C. Discard the supernatant and resuspend in 1 mL cold 1X Lysis Buffer. Incubate on ice for 10 minutes. Centrifuge at 10,000 x g for 10 minutes at 4°C. Carefully collect the supernatant and store on ice for immediate use. For longer term storage, freeze the lysate at -80°C for up to 1 month. Cell lysates may need to be further diluted in cold 1X Lysis Buffer before assaying.
- Adherent Cells: Carefully aspirate the culture media and wash once with the recommended volume of PBS (Table 2). Discard the supernatant and add the appropriate volume of cold 1X Lysis Buffer. Incubate on ice for 10 minutes. Transfer the lysate to a microcentrifuge tube. Centrifuge at 10,000 x g for 10 minutes at 4°C. Carefully collect the supernatant and store on ice for immediate use. For longer term storage, freeze the lysate at -80°C for up to 1 month. Cell lysates may need to be further diluted in cold 1X Lysis Buffer before assaying.

Culture Dish	96-well	48-well	24-well
PBS Wash Volume (µL/well)	200	400	800
1X Lysis Buffer (µL/well)	75	150	300

Table 2: Dispensing Volumes of Different Plate Formats

Assay Protocol

Important Note: Freshly prepare HK Enzyme standards each time the assay is performed. Maintain the HK Enzyme Standard, 10X HK Substrate, 10X Cofactor Mix, and 100X Enzyme Mix at 4ºC during assay preparation.

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each unknown sample replicate requires two paired wells, one to be treated with 10X HK Substrate (+Sub) and one without (-Sub) to determine background.

2. Add 50 µL of the HK Enzyme standards, samples or blanks to the 96-well microtiter plate.
3. Prepare the desired volume of Substrate Mixture (+Sub) according to Table 3 below, based on the number of tests to be performed. Maintaining all solutions at room temperature, add components **in the following sequence**:
   a. In a tube, add the appropriate volume of Assay Buffer.
   b. Next, add the corresponding volume of 10X HK Substrate and 10X Cofactor Mix.
   c. Finally, add the corresponding volume of Colorimetric Probe and 100X Enzyme Mix. Mix well and immediately use.

Assay Buffer (mL)	10X HK Substrate (mL)	10X Cofactor Mix (mL)	Colorimetric Probe (mL)	100X Enzyme Mix (µL)	Total Volume of Substrate Mixture (mL)	**# of Tests in 96-well Plate (50 µL/well)**
1	0.5	0.5	0.5	50	2.55	**50**
0.5	0.25	0.25	0.25	25	1.275	**25**
0.2	0.1	0.1	0.1	10	0.510	**10**

Table 3. Preparation of Substrate Mixture (+Sub)

4. Prepare the desired volume of Control Mixture (-Sub) according to Table 4 below, based on the number of tests to be performed. Maintaining all solutions at room temperature, add components **in the following sequence**:
   a. In a tube, add the appropriate volume of Assay Buffer.
   b. Next, add the corresponding volume of deionized water and 10X Cofactor Mix.

c. Finally, add the corresponding volume of Colorimetric Probe and 100X Enzyme Mix. Mix well and immediately use.

Assay Buffer (mL)	Deionized H_2O (mL)	10X Cofactor Mix (mL)	Colorimetric Probe (mL)	100X Enzyme Mix (µL)	Total Volume of Substrate Mixture (mL)	**# of Tests in 96-well Plate (50 µL/well)**
1	0.5	0.5	0.5	50	2.55	**50**
0.5	0.25	0.25	0.25	25	1.275	**25**
0.2	0.1	0.1	0.1	10	0.510	**10**

Table 4. Preparation of Control Mixture (-Sub)

5. Transfer 50 µL of the Substrate Mixture (+Sub) from step 3 above to all wells containing standards and to one half of the paired sample wells. Mix thoroughly.
6. Transfer 50 µL of the Control Mixture (-Sub) from step 4 above to the remaining half of the paired sample wells. Mix thoroughly.
7. Incubate at 37ºC for 15 minutes.
8. Read the absorbance of each microwell on a spectrophotometer using 450 nm as the primary wavelength.
9. Subtract the OD of the control wells (-Sub) from the OD of the substrate wells (+Sub) to obtain the Net OD for each sample replicate, before comparing to the standard curve.

Example of Results

The following figures demonstrate typical Hexokinase Activity Assay Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

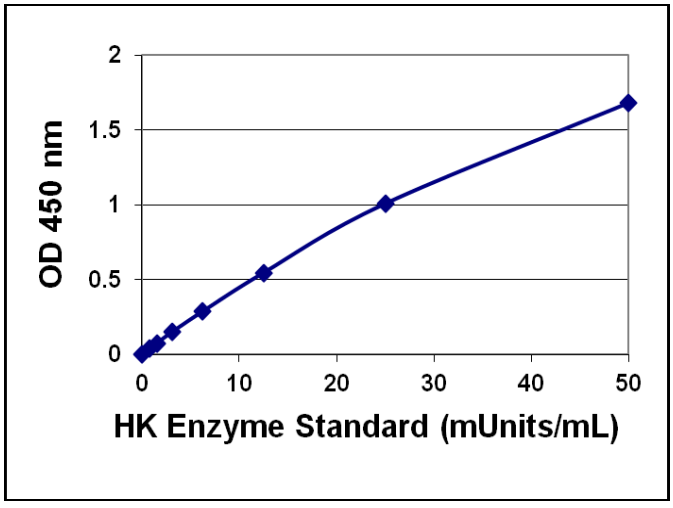

Figure 1: HK Enzyme Standard Curve. HK Enzyme standard curve was performed according to the Assay Protocol. Background has been subtracted.

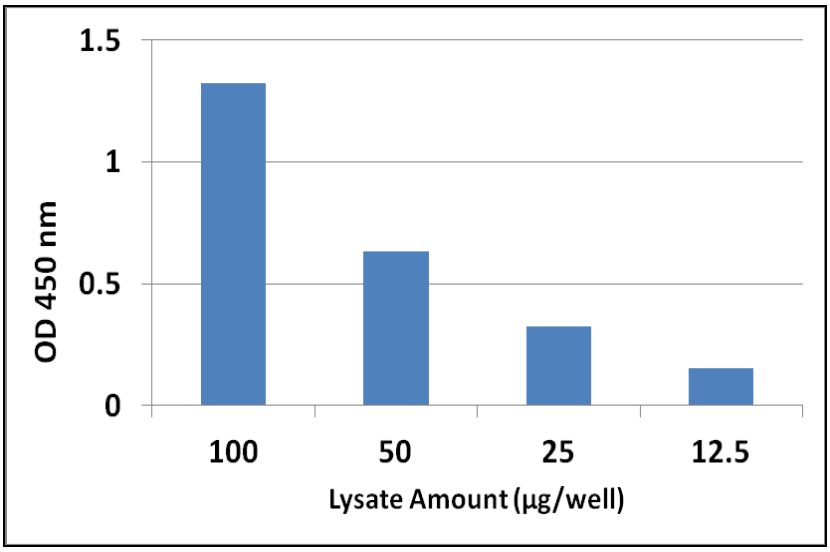

Figure 2: HK Activity of HEK-293 Lysate. HEK-293 lysate was prepared according to the Assay Protocol (total protein concentration was also determined). Background has been subtracted.

References

1. Anderson, M., Marayati, R., Moffitt, R., and Yeh, J. (2016) *Oncotarget* **8**, 56081-56094.
2. Meurer, F., Bobrownik, M., Sadowski, G., and Held, C. (2016) *Biochemistry* **55**, 5665-5674.
3. O'Sullivan, D., Kelly, B., and Pearce, E. (2016) *Cell Metab.* **24**, 198-200.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com