# Hexokinase Assay (HK)

*Cat. No. 8408*
*100 Tests in 96-well plate*

## Introduction

Hexokinase (HK), which phosphorylates glucose and generates glucose-6-phosphate for glycolysis, plays an important role in glucose metabolism. HK deficiency leads to severe human diseases such as X-linked muscular dystrophy and a rare autosomal recessive hemolytic anemia. On the other hand, increased HK activity is detected in various human tumors and is associated with metastasis. In ScienCell™ Hexokinase Assay kit, glucose is converted to glucose-6-phosphate by HK, the glucose-6-phosphate is oxidized by glucose-6-phosphate dehydrogenase to form NADPH, which can convert a nearly colorless probe to an intensely colored product, which exhibits maximum absorbance at 490nm, is proportional to the activity of HK in the sample.

## Kit Components

| Cat. No. | # of vials | Reagent | Quantity | Storage |
|---|---|---|---|---|
| 8408a | 1 | Assay buffer | 10 mL | 4°C |
| 8408b | 1 | HK standard (500X) | 20 µL | -80°C |
| 8408c | 1 | Substrate | 1.0 mL | -20°C |
| 8408d | 1 | Cofactor | 2.0 mL | -20°C |
| 8408e | 1 | Developer | 0.2 mL | -20°C |
| 8408f | 1 | Enzyme mix | 0.6 mL | -20°C |
| 8408g | 1 | NADP | 1.0 mL | -20°C |

## Product Use

Hexokinae Assay kit could measure HK activity of samples from cells and tissue. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

## Quality Control

Data from hexokinase assay of HK solutions with concentrations ranging from 0.8 to 50 mU/mL show a linear relationship between $OD_{490nm}$ and HK activity (Figure 1).

## Shipping

The kit would be shipped on dry ice.

**Sample Preparation**

1. HK standard (1X): dilute HK standard (500X) (8408b) in assay buffer (8408a) (1:500).

**Procedure (96-well plate)**

**A. Preparation of HK standard**

1. Add 15 µL of HK standard (1X) to 15 µL of assay buffer (8408a) to make a 30 µL solution of 100 mU/mL HK.
2. Obtain 7 test tubes, add 25 µL of assay buffer (8408a) into each tube and label them #1 through #7.
3. Add 25 µL of the 100 mU/mL into tube #1 and mix well to get the 50 mU/mL HK standard.
4. Transfer 25 µL of the 50 mU/mL HK standards from tube #1 to tube #2 and mix well to get the 25 mU/mL HK standard.
5. Repeat step 4 for tubes #3-6 to serially dilute the HK standards. Do not add any HK to tube #7, which serves as blank.
6. Obtain a 96-well test plate, prepare 2 replicates (A, B) of each HK standard by aliquoting 10 µL/well of each HK standard into duplicate wells of the 96-well test plate, according to the following plate format:

| | #1 | #2 | #3 | #4 | #5 | #6 | #7 |
|---|---|---|---|---|---|---|---|
| **A** | 50mU/mL | 25mU/mL | 12.5mU/mL | 6.25mU/mL | 3.125mU/mL | 1.56mU/mL | Blank |
| **B** | 50mU/mL | 25mU/mL | 12.5mU/mL | 6.25mU/mL | 3.125mU/mL | 1.56mU/mL | Blank |

**B. Preparation of test samples**

1. Cells or Tissues can be homogenized in 4 volumes of the assay buffer (8408a). Centrifuge the samples at 1000 ×g for 10 minutes to remove insoluble material. The soluble fraction may be assayed directly.
2. Endogenous NAD(H) or NADP(H) from cell or tissue extracts would generate background for the HK assay. To remove the NAD(H) or NADP(H) background, same amount of sample can be tested in the absence of substrate (8408c)
3. Samples should be serial diluted to make sure the readings are within the standard curve range. Prepare test samples to a final volume of 10 µL/well on the 96-well flat bottom plate.

**C. Working reagent preparation and measurements**

1. For each well of reaction, prepare working reagent by mixing 42 µL assay buffer (8408a), 10 µL substrate, (8408c), 20 µL cofactor (8408d) and 2 µL developer (8408e), 6 µL enzyme mix (8408f), 10 µL NADP (8408g). Without adding substrate (8408c) into the well containing test samples for control.
2. Add 90 µL of working reagent mix into each well of the 96-well plate containing HK standard, test samples, blank and test samples for control. Incubate for 60 minutes at room temperature in dark.
3. Read the absorbance at 490 nm with an ELISA plate reader.

## D. Calculations

1. Average the $OD_{490nm}$ of duplicate wells of each HK standard, test sample and blank. Subtract the $OD_{490nm}$ value of the blank from the $OD_{490nm}$ values obtained with all other standard and samples to get $\Delta OD_{490nm}$ value.
2. Based on the calibrated $\Delta OD_{490nm}$ of the HK standard, make a standard curve by plotting $\Delta OD_{490nm}$ as a function of HK activity (See Figure 1 for a typical standard curve). Determine the equation and $R^2$ value of the trend line.
3. For samples requiring control without substrate, subtract the $\Delta OD_{490nm}$ without substrate value from the $\Delta OD_{490nm}$ with substrate value and use this $\Delta\Delta OD_{490nm}$ value to determine the sample HK activity from the standard curve.
4. Suppose the equation of the trend line of the standard curve is $y = Ax + B$, calculate the HK activity of test samples as follows:

$$[HK] = \frac{\Delta\Delta\ OD_{490nm}}{A}$$

**Unit definition**: One unit will generate 1.0 µmole of NADH per minute at pH 8.5 at 25 ºC.

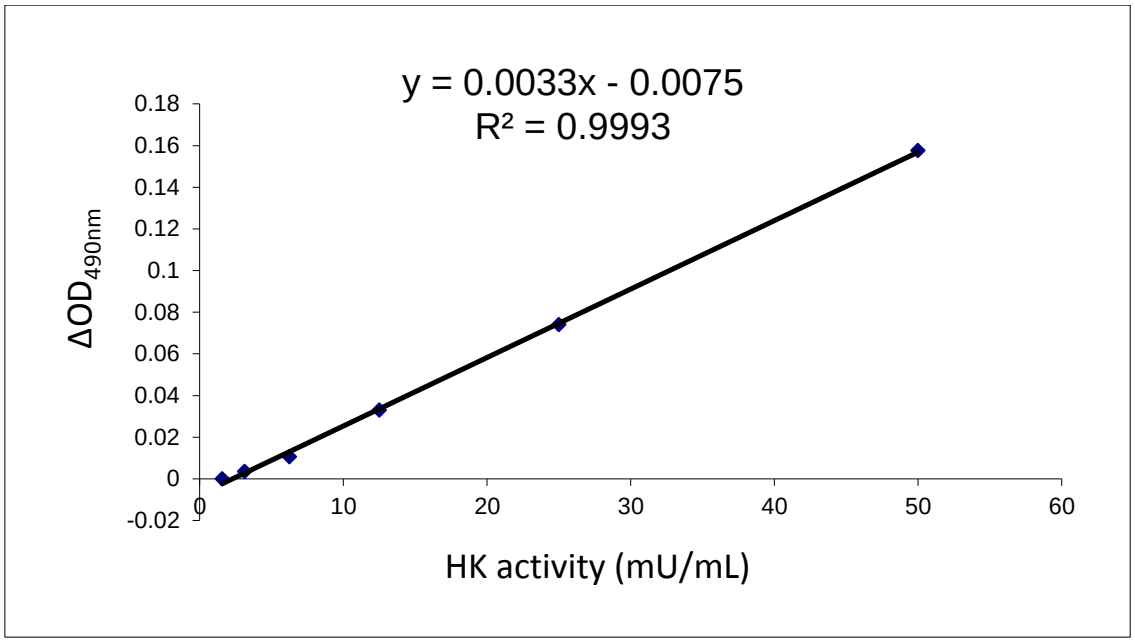


Figure1. A typical HK standard curve measured by ScienCell™ Hexokinase Assay kit