Rev. 0

Hexokinase Activity Assay Kit-Fluorometric (HK-FL)

Catalog #8798

100 Tests in 96-well plate

Product Description

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. Conversely, increased HK activity is deleterious and is associated with cancer and metastasis. In ScienCell™ Hexokinase Activity Assay kit-Fluorometric (HK-FL), glucose is converted to glucose-6-phosphate by HK, the glucose-6-phosphate is oxidized by glucose-6-phosphate dehydrogenase to form NADPH. In a coupled reaction, the generated NADPH reduces a probe, producing a highly fluorescent product. The fluorescence intensity, measured at an excitation wavelength of 560 nm and an emission wavelength of 590 nm, is directly proportional to the activity of hexokinase in the sample. The assay demonstrates a linear response up to 50 mU/mL, with a detection limit as low as 0.8 mU/mL.

Kit Components

Cat#	# of vials	Reagent	Quantity	Storage
8798a	1	Assay buffer	10 mL	4°C
8798b	1	HK standard (50 U/mL)	15 µL	-80°C
8798c	1	Substrate	1.0 mL	-20°C
8798d	1	Cofactor	2.0 mL	-20°C
8798e	1	Developer	0.2 mL	-20°C
8798f	1	Enzyme mix	0.6 mL	-20°C
8798g	1	NADP	1.0 mL	-20°C

Material Supplied by User

Fluorescent microplate reader (excitation 530-560/emission 580-600)
96-well plate

Product Use

HK-FL can be used for rapid and sensitive measurement of HK activity in variety of samples such as cells and tissues. HK-FL is for research use only and is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Quality Control

HK-FL is applied to serially diluted Hexokinase Standards with concentrations ranging from 3-50 mU/mL and is used to generate a standard curve (Figure 1).

Shipping

All components are shipped on dry ice.

Procedure (96-well plate)

A. Preparation of HK standard

1. Dilute HK standard (50 U/mL) (cat# 8798b) 250X in assay buffer (cat #8798a) to make 200 mU/mL stock. For example, mix 2 µL of HK standard with 498 µL of Assay Buffer to make 200 mU/mL hexokinase.
2. Add 15 µL of HK standard (1X) to 15 µL of assay buffer to make a 30 µL solution of 100 mU/mL HK.
3. Obtain 7 test tubes, add 25 µL of assay buffer into each tube and label them #1 through #7.
4. Add 25 µL of the 100 mU/mL into tube #1 and mix well to get the 50 mU/mL HK standard.
5. 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.
6. Repeat step 5 for tubes #2-6 to serially dilute the HK standards. Do not add any HK to tube #7, which serves as blank.
7. 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. 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-FL assay. To remove the NAD(H) or NADP(H) background, same amount of sample can be tested in the absence of substrate (cat# 8798c).
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 (cat# 8798a), 10 µL Substrate (cat# 8798c), 20 µL Cofactor (cat# 8798d), 2 µL Developer (cat# 8798e), 6 µL Enzyme Mix (cat# 8798f) and 10 µL NADP (cat# 8798g). Without adding substrate (cat# 8798c) into the well containing test samples for control.
   Note: *The sensitivity of the assay can be increased by reducing the Developer concentration in the 2X working reaction mixture. For example, add 1 µL of Developer per well and add 43 µL Assay buffer. Note that this change decreases the maximum attainable signal.*
2. Add 90 µL of working reagent mix into each well of the 96-well plate containing HK standard, test samples and blank.
3. Incubation time depends on the Hexokinase activity in the samples. Longer incubation time may be required if activity in the sample is low. We recommend measuring fluorescence in kinetic mode, and choosing the time that STD is linear.
4. Alternatively, incubate the plate for 10-30 minutes at room temperature in the dark and measure the fluorescence intensity (RFU) at excitation 530-560 nm, emission 580-600 nm.
5. If the sample's fluorescence intensity surpasses that of the 50 mU/mL hexokinase standard, dilute the sample with purified water and rerun the assay.

D. Calculations

1. Average the RFU reading of replicate wells of each hexokinase standard, test sample and blank.
2. Subtract the average RFU value of the blank from the average RFU values obtained with all other standards and samples
3. Make a standard curve (shown in Figure 1) using the corrected RFU values of the hexokinase against standard concentration. Determine the equation and R^2 value of the trend line.
4. Calculate the hexokinase concentration of test samples by using the equation for the trend line of the standard curve (y = Ax + B):

$$\text{Hexokinase(mU/mL)} = \frac{\text{calibrated } RFU}{\text{A}} \times \text{DF}$$

DF= Dilution factor

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

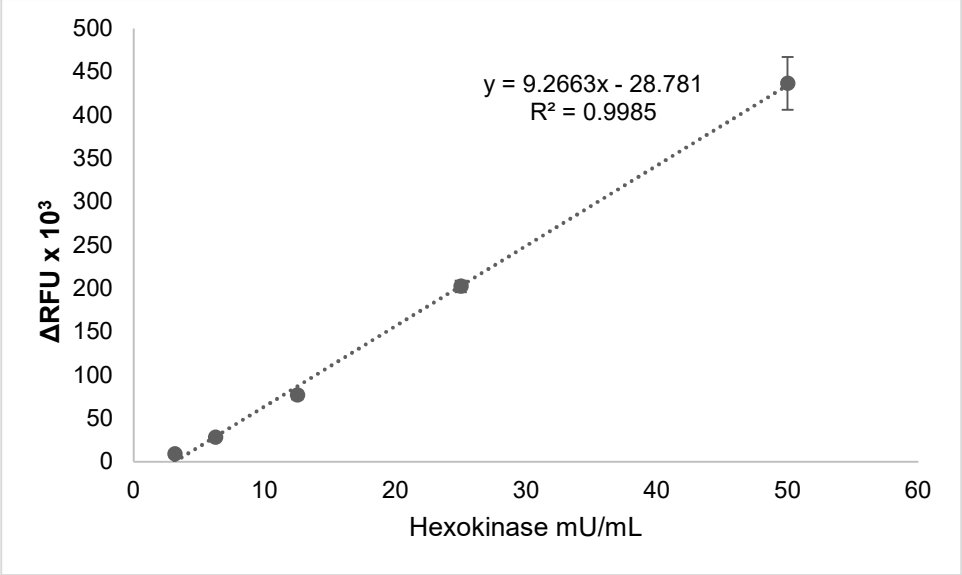

Figure1. A typical HK standard curve measured at 20 minutes by ScienCell™ Hexokinase Activity Assay-Fluorometric.