Tribioscience

Hexokinase Activity Fluorometric Assay (TBS2107F, 100Assay, Store at -20℃)

DESCRIPTION

Hexokinase (HK, 6-Phosphate Glucose Kinase, ATP: D-Hexose 6-Phosphotransferase, ATP-dependent Hexokinase) plays an important role in glucose metabolism. Hexokinase deficiency leads to diseases such as X-linked muscular dystrophy and rare autosomal recessive hemolytic anemia. On the other hand, increased hexokinase activity is detected in various human tumors and is associated with metastasis.

Tribioscience's Hexokinase Activity Fluorometric Assay kit provides a flexible, accurate, sensitive, and time-saving approach for detecting HK activity in a wide variety of samples. In this assay, HK converts glucose into glucose-6-Phosphate(G6P), which in turn undergoes a series of reactions and induces the Fluorometric-Probe to generate intense fluorescence product at 585 nm.

FEATURES

- **Flexible:** Suitable for 96-well or 384 well plate.
- **Accuracy**: Absorbance measurement is proportional to the HK concentration.
- **Sensitive:** Can detect as low as 0.5mU/mL.
- **Time-saving**: Just incubate and read out style.

Kit Components and Storage for 100 Assays

Part Name	Part Size	Part number
Assay Buffer	12 mL	TBS2107F-01
HK standard (10 U/mL)	50 µL	TBS2107F-02
HK Substrate	1.0 mL	TBS2107F-03
HK Enzyme Mix	1.0 mL	TBS2107F-04
HK Probe	1.0 mL	TBS2107F-05
Cofactor	1.0 mL	TBS2107F-06

APPLICATIONS

- Hexokinase activity in different samples like cells and tissues.

DIRECTIONS FOR USE

Sample Preparation

1. Cells or Tissues are homogenized in 3 volumes of the assay Buffer (TBS2107F-01).
2. Centrifuge the samples at 2000xg for 10 min at 4°C.
3. Take the supernatant to new tube on the ice. If not assaying the same day, freeze the samples at -80°C for a month.

Assay Procedures

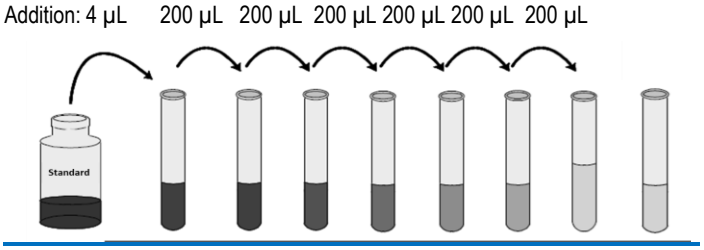
1. Standard Preparation:

1.1 Label test tubes as #1 through #8. Pipet 396 µL of 1x Assay Diluent into tube #1, and 200 µL into tubes #2 to #8 as Fig, 1. Diagram.

1.2 Add 4 µL of the HK Standard stock (10 U/mL) to tube #1 and mix to make standard concentration of 100 mU/mL.

1.3 Make 2x serial dilutions of the standard using the Tube#1(100mU/mL standard solution) from Tube #2 through #7 with sequential transfer of 200 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 0 mU/mL. Tube# 8 is Standard 8 (0 pg/mL).

Fig.1 Diagram for HK standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	396	200	200	200	200	200	200	200
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	4	200	200	200	200	200	200	0
Conc (mU/mL)	100	50	25	12.5	6.25	3.125	1.56	0

2. Load samples:
Add 50 µL Standard, sample, and control to each well. (Note: recommend running a pilot study to determine the optimal concentration of sample within the assay standard curve range).

3. Assay Working reagent Preparation:
Mixing the below reagent for each well:
- 37.8 µL Reaction Buffer (TBS2107F-01)
- 1 µL substrate stock solution (TBS2107F-03).
- 1 µL Enzyme Mix (TBS2107F-04)
- 10 µL Enzyme Probe (TBS2107F-05)
- 0.2 µl Cofactor Stock solution (TBS2107F-06).

4. Add 50µL of above assay working reagent to each well containing the standards, controls, and samples.

5. Incubate the reactions. Incubate at room temperature for 30 ~ 60 minutes protected from light.
6. Read fluorescence intensity at Ex/Em: 503/580 nm.

For 384 well plate, the reagents should be reduced to half.

Calculation

Subtract the blank value (0mU/mL Standard) from the standard values and plot the ΔFU against standard concentrations. Determine the slope and calculate the hexokinase activity of the Sample using the equation obtained from the linear regression of the standard curve.

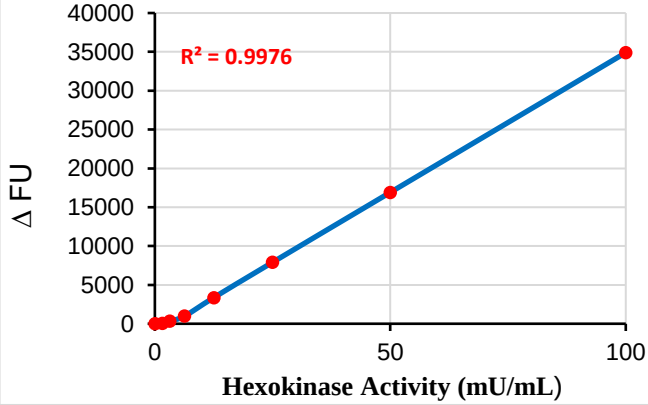
Hexokinase Activity (mU/mL) = N x (Rsample- Rblank)/Slope

Where: Rsample and Rblank are optical density readings of the sample and blank, respectively. N is the sample dilution factor.

Typical Standard Curve

The typical standard curve shown in Fig. 2. is only for reference. It cannot be used for hexokinase activity analysis.

Fig. 2 Hexokinase Activity FLorometric Assay



RELATED PRODUCTS

Hexokinase Activity Colorimetric Assay (TBS2107C)
6-Phosphogluconate Colorimetric Assay (TBS2089C)
6-Phosphogluconate Dehydrogenase Activity Colorimetric Assay (TBS2093C)
Glucose-6-phosphate dehydrogenase Activity Colorimetric Assay (TBS2102C)
Resazurin Cell Viability (TBS2001)
LDH Cytotoxicity Assay (TBS2002)
Catalase Assay (TBS2006)
ATP Colorimetric/ Fluorometric Assay (TBS 2010)
ADP Colorimetric/Fluorometric Assay (TBS3020)
CCK-8 Cell Viability Assay (TBS2022)
Caspase-3 Colorimetric Assay (TBS2030)
Cytochrome c Reductase Activity Assay (TBS2116)
AOPI Viability Assay for Flow Cytometry (TBS2069

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