Tribioscience

Hexokinase Activity Colorimetric Assay (TBS2107C, 100Assay, Store at -20°C)

DESCRIPTION

Hexokinase (EC 2.7.1.1, HK, 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 Colorimetric 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), then undergoes a series of reactions and induces the Colorimetric-Probe to generate a strong colored product detected at 460 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 7 mU/mL.
- **Time-saving**: Just incubate for 30 minutes and read out.

Kit Components and Storage for 100 Assays

Part Name	Part Size	Part number
Assay Buffer	10 mL	TBS2107C-01
HK standard (2.5 U/mL)	0.1mL	TBS2107C-02
HK Substrate (100x)	0.1 mL	TBS2107C-03
HK Probe (5x)	1.2 mL	TBS2107C-04
Cofactor	20 µL	TBS2107C-05
HK Enzyme mix	0.1 mL	TBS2107C-06

APPLICATIONS

- Hexokinase activity in different samples like cells and tissues.

Sample Preparation

1. Cells or Tissues are homogenized in 3 volumes of the assay Buffer (TBS2107C-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

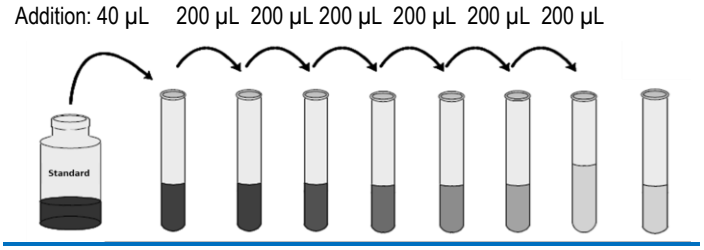
1. Standard Preparation:

1.1 Label test tubes as #1 through #8. Pipet 360 µL of 1x Assay Buffer into tube #1, and 200 µL into tubes #2 to #8 as Fig, 1. diagram.

1.2 Add 40 µL of the HK Standard stock (2500 mU/mL) to tube #1 and mix to make standard concentration of 250 mU/mL.

1.3 Make 2x serial dilutions of the standard using the Tube#1(250 mU/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 250, 125, 62.5, 31.25, 15.6, 7.8 and 3.9 mU/mL. Tube# 8 is Standard 8 (0 mU/mL).

Fig.1 Diagram for HK standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	360	200	200	200	200	200	200	200
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	40	200	200	200	200	200	200	0
Conc (mU/mL)	250	125	62.5	31.25	15.6	7.8	3.9	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. Preparation of reaction mix: mixing the below reagents for 100 assays, total volume 5 mL.

Reaction Mix	Volume
Assay Buffer	3890 µL
HK Enzyme mix	50 µL
HK Substrate	50 µL
Cofactor	10 µL
HK Probe	1000 µL

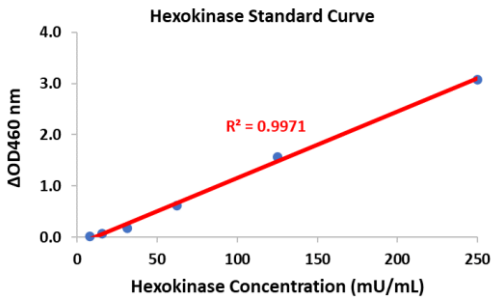
4. Add 50 µL of reaction mix to each well containing the standards, controls, and samples.

5. Incubate at room temperature for 30 minutes, protected from light.

6. Measure OD460 nm.

7. Calculate the hexokinase activity with the Hexokinase standard curve.

Typical Hexokinase standard curve: Y=AX + B
Y is ΔOD460: the OD460 subtract the blank.
X is the Hexokinase concentration: mU/mL.
A is the slope and B is the Y-intercept.



Hexokinase activity (mU/mL) = (ΔOD460nm - B)* N /A
ΔOD460 is the OD460 subtract the blank.
N: dilution factor.

Note: avoid the reducing agents (β-mercaptoethanol, dithiothreitol (DTT)) and ketonic monosaccharides, such as fructose in the assay as these can generate false-positive signals.

RELATED PRODUCTS

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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