TECHNICAL MANUAL

Hexokinase (HK) Activity Assay Kit

- **SKU CODE:** MAES0242
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate at 37°C for 10 minutes
- Measure absorbance at 450 nm

2. Intended use

This kit can measure hexokinase (HK) activity in animal tissue and cell samples.

3. Detection principle

Hexokinase (HK) is a key enzyme in glucose decomposition that exists in different species. It is an enzyme that catalyzes the phosphorylation of hexose to generate hexose phosphate, and is also one of the key enzymes in carbohydrate metabolism. HK converts glucose to glucose 6-phosphate through enzymatic catalysis. Meanwhile, NAD+ is reduced to NADH, which under the action of hydrogen transmitter, transfers electrons to WST-8 to produce a yellow product. The activity of HK can be calculated by measuring the change in absorbance value at 450 nm.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	25 mL × 1 vial	-20°C, 12 months
Reagent 2	Substrate	Powder × 1 vial	-20°C, 12 months, shading light
Reagent 3	Enzyme Reagent	Powder × 1 vial	-20°C, 12 months, shading light
Reagent 4	Chromogenic Agent	3 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	0.8 mmol/L Standard	3.2 mL × 1 vial	-20°C, 12 months, shading light

Item	Component	Size (96 T)	Storage
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Centrifuge, 37°C Incubator, Microplate reader (440-450 nm, optimum wavelength: 450 nm)

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For small volume reagents, please centrifuge before use to ensure sufficient reagent recovery.

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate stock solution:** Dissolve one vial of substrate with 12 mL of buffer solution, mix well to dissolve. Store aliquots at -20°C for 2 weeks protected from light.
3. **Preparation of enzyme stock solution:** Dissolve one vial of enzyme reagent with 12 mL of buffer solution, mix well to dissolve. Store aliquots at -20°C for 2 weeks protected from light.
4. **Preparation of enzyme working solution:** Before testing, prepare sufficient enzyme working solution according to the number of test wells. For example, prepare 300 µL of enzyme working solution by mixing 150 µL of substrate working solution and 150 µL of enzyme stock solution. The enzyme working solution should be prepared fresh. Store at 2-8°C for 12 hours protected from light.
5. **Preparation of sample reaction solution:** For each well, prepare 220 µL of sample reaction solution by mixing 200 µL of enzyme working solution and 20 µL of chromogenic agent. The sample reaction solution should be prepared fresh. Store at 2-8°C for 12 hours protected from light.
6. **Preparation of control reaction solution:** For each well, prepare 220 µL of control reaction solution by mixing 200 µL of double distilled water and 20 µL of

chromogenic agent. The control reaction solution should be prepared fresh. Store at 2-8°C for 12 hours protected from light.

7. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 0.8 mmol/L standard solution with double distilled water to create a serial concentration series. The recommended dilution gradient is as follows: 0, 0.16, 0.32, 0.4, 0.48, 0.56, 0.64, 0.8 mmol/L.

7. Sample preparation

Sample preparation

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 µL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000×g for 10 minutes at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Cell (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation 5×10^6 cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 5×10^6 cells in 200 µL normal saline (0.9% NaCl) with an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10,000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

10% Mouse spleen tissue homogenate: 1

10% Mouse liver tissue homogenate: 1

10% Mouse kidney tissue homogenate: 1

10% Mouse heart tissue homogenate: 2-6

10% Mouse brain tissue homogenate: 1

10% Rat spleen tissue homogenate: 1

10% Rat lung tissue homogenate: 1

10% Rat liver tissue homogenate: 1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

The powder must be completely dissolved while preparing the working solution.

9. Operating steps

1. Standard well: Add 10 μL of standard solution with different concentrations to the corresponding wells. Control well: Add 10 μL of sample to the corresponding wells. Sample well: Add 10 μL of sample to the corresponding wells.
2. Add 220 μL of sample reaction solution to standard wells and sample wells. Add 220 μL of control reaction solution to control wells.
3. Incubate at 37°C for 10 minutes.
4. Measure the OD value of each well at 450 nm with microplate reader.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absolute OD value.

3. Plot the standard curve by using absolute OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

Tissue and cell samples:

Definition: The amount of HK in 1 g tissue or cell protein per 1 minute that hydrolyzes the substrate to produce 1 μmol glucose-6-phosphate at 37°C is defined as 1 unit.

HK activity (U/g_{prot}) = (ΔA_450 - b) ÷ a ÷ T × 1000 ÷ C_{pr} × f

[Note]

y: $OD_{Standard}$ – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_450: $OD_{Sample} - OD_{Control}$.

T: The time of reaction, 10 min.

C_{pr}: The concentration of protein in sample, g_{prot}/L.

f: Dilution factor of sample before test.

1000*: 1 mmol = 1000 μmol.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three mouse heart tissue samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	12.40	25.50	32.00
%CV	3.6	3.2	3.1

Inter-assay Precision

Three mouse heart tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	12.40	25.50	32.00
%CV	8.7	9.5	9.1

Recovery

Three samples of high, middle, and low concentrations were tested with 6 replicates for each concentration to obtain an average recovery rate of 99%.

Recovery

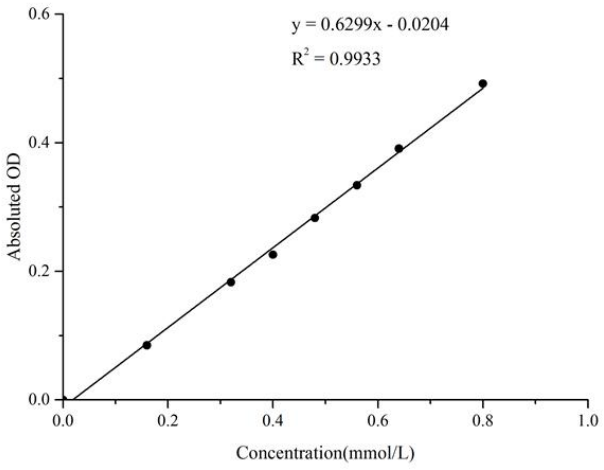
	standard 1	standard 2	standard 3
Expected Conc. (mmol/L)	0.28	0.45	0.62
Observed Conc. (mmol/L)	0.3	0.4	0.6
Recovery rate (%)	101	95	101

Sensitivity

The analytical sensitivity of the assay is 5.93 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



Standard curve

Concentration (mmol/L)	Average OD	Absolute OD
0	0.049	0
0.16	0.132	0.085
0.32	0.232	0.183
0.4	0.275	0.226
0.48	0.332	0.283
0.56	0.383	0.334
0.64	0.440	0.391
0.8	0.541	0.492

12. Appendix II Example Analysis

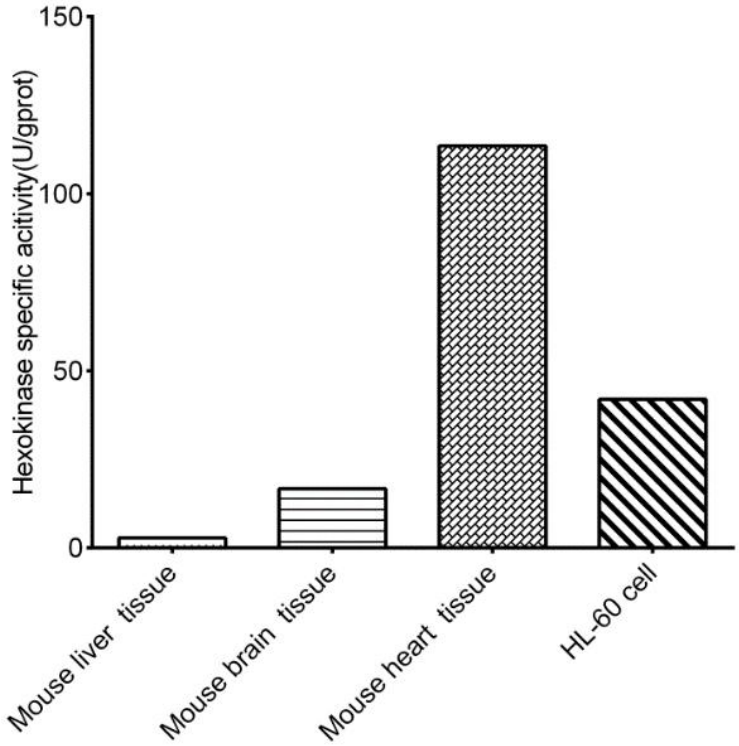
Example analysis:

For mouse liver tissue, take 10 μL of 10% mouse liver tissue homogenate, and carry out the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.6299 x – 0.0204, the average OD value of the control is 0.157, the average OD value of the sample is 0.298, the concentration of protein in sample is 9.45 g_{prot}/L, and the calculation result is:

HK activity (U/g_{prot}) = (0.298 – 0.157 + 0.0204) ÷ 0.6299 ÷ 10 × 1000 ÷ 9.45 = 2.71 U/g_{prot}

Detect 10% mouse liver tissue homogenate (protein concentration: 9.45 g_{prot}/L), 10% mouse brain tissue homogenate (protein concentration: 3.93 g_{prot}/L), 10% mouse heart tissue homogenate (protein concentration: 6.45 g_{prot}/L, diluted 5 times) and HL-60 cells (protein concentration: 0.79 g_{prot}/L) according to the protocol. The results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.