Product Information

Hexokinase Colorimetric Assay Kit

I. Kit Contents:

Components	K2179-100 100 assays	Cap Color	Part Number
HK Assay Buffer	25 ml	WM	K2179-C-1
HK Substrate	1 ml	Blue	K2179-C-2
HK Coenzyme (Lyophilized)	1 vial	Purple	K2179-C-3
HK Enzyme Mix (Lyophilized)	1 vial	Green	K2179-C-4
HK Developer (Lyophilized)	1 vial	Red	K2179-C-5
NADH Standard (Lyophilized)	1 vial	Yellow	K2179-C-6
HK Positive Control (Lyophilized)	1 vial	Orange	K2179-C-7

II. Introduction:

Hexokinases phosphorylates glucose to generate glucose-6-phosphate, the initial step in glucose metabolism. HK1 and HK2 are two subtypes of mitochondrial hexokinases which are also involved in antiapopotic and cell survival. Hexokinase deficiency leads to X-linked muscular dystrophy and autosomal recessive hemolytic anemia. Increased hexokinase activity is detected in tumors and associated with metastasis. Early detection of abnormal hexokinase activity is crucial for diagnosis, prediction and treatment of the disease. In this Hexokinase Colorimetric Assay Kit, hexokinase converts glucose into glucose-6-phosphate which is further oxidized by glucose-6-phosphate dehydrogenase to form NADH, and in turn reduces a colorless probe to a colored product with intense absorbance at 450 nm. The assay offers an easy and sensitive way for measuring hexokinase activity. The detection limit can be as low as 0.1 mU/well.

III. Application:

Measurement of Hexokinase activity in various tissues/cells.

Analysis of glucose metabolism and cell signaling in various cell types.

Screening anti-diabetic drugs.

IV. Sample Type:

Serum.

Animal tissues: Liver, Heart, Kidney etc.

Cell culture: Adherent or suspension cells.

V. User Supplied Reagents and Equipment:

96-well plate with flat clear bottom.

Multi-well spectrophotometer (ELISA reader).

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm all buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

HK Coenzyme: Reconstitute with 220 µl HK Assay Buffer to generate 0.2 M solution. Store at -20°C. Use within two months. Keep on ice while in use.

HK Enzyme Mix: Reconstitute with 220 µl HK Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months. Keep on ice while in use.

HK Developer: Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.

NADH Standard: Reconstitute with 400 µl dH_2O to generate 1.25 mM (1.25 nmol/µl) NADH Standard solution. Store at -20°C. Use within two months. Keep on ice while in use.

HK Positive Control: Reconstitute with 100 µl HK Assay Buffer and mix thoroughly. Aliquot and store at -20°C.

VIII. Hexokinase Assay Protocol:

1. NADH Standard Curve: Add 0, 2, 4, 6, 8 and 10 µl of 1.25 mM NADH Standard into a series of wells in duplicate in 96 well plate to generate 0, 2.5, 5.0, 7.5, 10 and 12.5 nmol/well of NADH Standard. Adjust volume to 50 µl/well with HK Assay Buffer.

2. Sample Preparation: Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 200 µl ice cold HK Assay Buffer for 10 minutes on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add 1 - 50 µl sample (40 µg) per well, adjust final volume to 50 µl with HK Assay Buffer. Prepare a parallel sample well as the background control to avoid interference from the NADH in the sample. Note: For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range.

3. HK Positive Control: Dilute Positive control solution 1:99 in HK Assay Buffer. Use 1-10 µl of diluted Positive Control into the desired well(s) and adjust the final volume to 50 µl with HK Assay Buffer.

4. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	Background Control Mix
HK Assay Buffer	34 µl	44 µl
HK Enzyme Mix	2 µl	2 µl
HK Developer	2 µl	2 µl
HK Coenzyme	2 µl	2 µl
HK Substrate	10 µl	---

Add 50 µl of the reaction mix to each well containing the Standard, Positive Control and test samples and 50 µl of background control mix to each well containing the background control sample. Mix well.

5. Measurement: Incubate for 20-60 min at room temperature and measure OD450nm. Note: Incubation time depends on the Hexokinase activity in the samples. We recommend measuring the OD in a kinetic mode, and choose two time points (T1 & T2) in the linear range to calculate the hexokinase activity of the samples. The NADH standard curve can read in endpoint mode (i.e., at the end of incubation time).

6. Calculation: Subtract the 0 standard reading from all standard readings. Plot the NADH standard curve. Correct sample background by subtracting the value derived from the background control from all sample readings. Calculate the hexokinase activity of the test sample: $\Delta OD = A2 - A1$. Apply the ΔOD to the NADH standard curve to get B nmol of NADH generated by hexokinase during the reaction time ($\Delta T = T2 - T1$).

Sample Hexokinase activity = $B/(\Delta T \times V) \times$ Dilution Factor = nmol/min/ml/ = mU/ml

Where: B is the NADH amount from standard curve (nmol).

ΔT is the reaction time (min).

V is the sample volume added into the reaction well (ml).

Unit Definition: One unit of hexokinase is the amount of enzyme that will generate 1.0 µmol of NADH per min at pH 8 at room temperature.

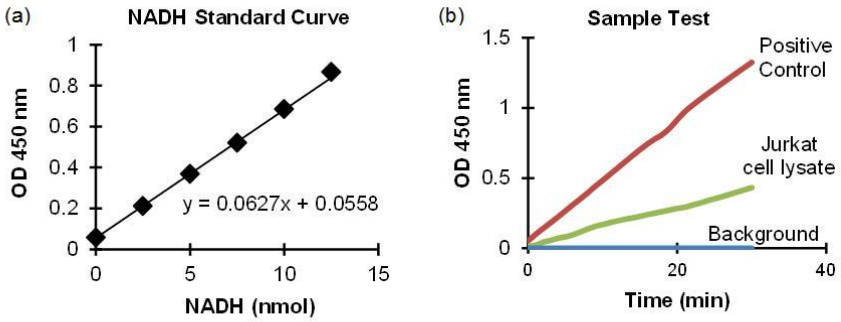

Figure 1: NADH standard curve (a). Hexokinase activity in Positive Control and Jurkat cell lysate (40 µg) (b). Assays were performed following kit protocol.

Frequently Asked Questions

1. In measuring D-Glucose-rich sample (such as muscle), is there a possibility that the background control shows slightly higher values than the sample itself?

If there is Higher NADH in the muscle sample than D-glucose, then background can be equivalent or higher. Since OD is measured in a kinetic mode, time points can be chosen within the linear regime such that background values are lower than D-glucose reactions. Sample volume in the well can also be optimized such that very high (~ 50ul) volumes are not used. Low volumes will prevent saturation and will allow better distinction between sample and background.

2. What time should the standard data be read at?

Standards should be read at end-point (at the highest timepoint for the sample readings).

3. Do hemolysed pieces affect the assay?

Any particulate matter in suspension/turbidity affects absorbance measurements. Hemoglobin color or hemolysis in the sample can influence the OD measurements but typically OD 450nm is the brown/yellow range and hence red color does not influence much. I would suggest diluting the sample, removing the hemolyzed chunks and then using the sample.

4. Does this kit measure activity of HK-1 or HK-2 or other isoforms also? What about muscle tissue? What is the source of the positive control enzyme?

This kit is based on function of the Hexokinase enzyme (conversion of glucose to glucose-6-phosphate is measured). This kit does not distinguish between the isoforms. HKI, HKII and HKIII have low Km, while HKIV has 100 fold high Km. Hence it is very likely that you will measure hexokinase activity from I, II and III and small contribution if any from HK IV depending on the relative amounts of these isoforms in your sample. HK-II is the predominant form in adipose and muscle cells. HKII is insulin-sensitive. So if the samples are from adipose tissue or muscle you will measure HK-II activity. Our positive control is HK-II from Bacillus sp.

5. The substrate of G6PDH should be NADP, not NAD, right ?

We use NAD and not NADP for G6PDH. G6PDH enzyme can work with both NAD and NADP depending on the species origin (sometimes there is absolute specificity like the G6PDH from Aspergillus does not work with NADP at all, but our enzyme works with both NAD and NADP). We believe the Km is better with NAD and hence we use NAD in our assay.

6. Can I use other hexose-sugars as substrates, eg Fructose?

This kit assays hexokinase activity in a variety of samples. Theoretically hexokinases in general can phosphorylate hexose sugars including fructose which is then phosphorylated to fructose-6-P. Nevertheless, the primary substrate is glucose for these enzymes in mammalian cells. Hexokinase IV (fastest hexokinase) is actually a glucokinase meaning it acts on glucose to form G6P. It might be possible to use fructose as a substrate to test since NADH formed after phosphorylation reacts with the probe to generate color. But this depends on whether the sample has fructokinase activity or is mainly a glucokinase. The substrate provided in the kit is D-glucose.

7. What is the activity in the positive control?

The positive control is a purified enzyme and the specific activity changes from lot to lot. The positive control is provided to be used as a benchmark sample to make sure all components of the kit are working. The positive control is not to be used to compare activity in the sample.

8. Can frozen samples be used with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

9. Is it possible to use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

10. Can alternate buffers be used for sample preparation (cell lysis, sample dilutions etc)?

Our assay buffers are optimized for the reactions they are designed for. They not only contain some detergents for efficient lysis of your cells/tissue, but also contain some proprietary components required for the further reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com