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ab211107 Hexokinase Inhibitor Screening Kit (Colorimetric)

For the rapid, sensitive and accurate screening of potential Hexokinase inhibitors.

This product is for research use only and is not intended for diagnostic use.

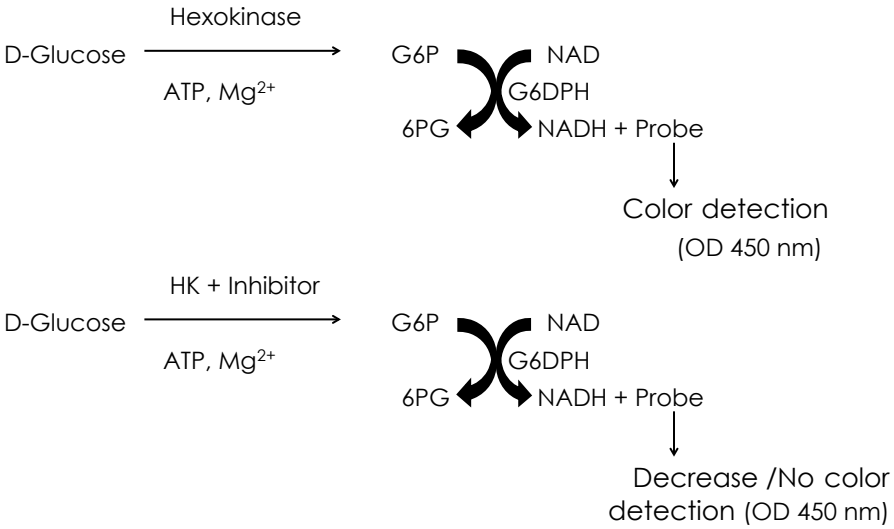
PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

Hexokinase Inhibitor Screening Kit (Colorimetric) (ab211107) provides a rapid, simple and adaptable test for high-throughput screening of Hexokinase (HK) inhibitors within 30 minutes. Glucose is converted to glucose-6-phosphate by hexokinase; the glucose-6-phosphate is then oxidized by glucose-6-phosphate dehydrogenase to form NADH, which reduces a colorless probe to a colored product with strong absorbance at 450 nm. In the presence of a Hexokinase inhibitor, the reaction is impeded/abolished resulting in decrease or total loss of absorbance.

This simple and high-throughput adaptable assay kit can be used to screen, study or characterize potential inhibitors of Hexokinase.



Hexokinase (HK, 6-Phosphate glucose kinase, ATP-dependent hexokinase, EC 1.1.1.49) is responsible for phosphorylating hexoses to form hexose phosphate. Hexokinases play an important role in glucose metabolism, as glucose is the most important substrate of hexokinases. In mammals, there are four HK isoforms. HK-I, HK-II, and HK-III are referred as "low Km" because of their high affinity for glucose (Km < 1mM), while HK-IV (also known as Glucokinase) has a Km for glucose 100-fold higher and can only phosphorylate glucose when the substrate concentration is high enough.

2. Protocol Summary

Screening compound & controls preparation

Enzyme and substrate solution preparation

Add enzyme solution to wells.
Incubate for 5 minutes at 25°C

Add substrate solution to wells

Measure absorbance at OD450 nm in kinetic mode
for 5 - 30 minutes at 25°C

**For kinetic mode detection, incubation time given in this summary is for guidance only*

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: Reconstituted components are stable for 2 months.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer LX/HK Assay Buffer	25 mL	-20°C	4°C/ -20°C
HK Substrate	1 mL	-20°C	-20°C
HK Coenzyme/HK Coenzyme (25 mg)	1 vial	-20°C	-20°C
Development Enzyme Mix IX/HK Converter (10 U)	1 vial	-20°C	-20°C
Developer Solution III/HK Developer (30 mg)	1 vial	-20°C	-20°C
HK Positive Control/Hexokinase (0.5 U)	1 vial	-20°C	-20°C
Hexokinase Inhibitor/HK Inhibitor Control (40 µmole)	1 vial	-20°C	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 450 nm
- MilliQ water or other type of double distilled water (ddH_2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well clear plate with flat bottom, preferably white

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer LX/HK Assay Buffer (25 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C or -20°C.

9.2 HK Substrate (1 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C.

9.3 HK Coenzyme/HK Coenzyme (lyophilized, 25 mg):

Reconstitute HK Coenzyme in 220 µL of ddH_2O. Pipette up and down to dissolve completely. Aliquot coenzyme so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.4 Development Enzyme Mix IX/HK Converter (lyophilized, 10 U):

Reconstitute Development Enzyme Mix IX/HK Converter in 220 µL of Assay Buffer LX/HK Assay Buffer by pipetting up and down. Aliquot Development Enzyme Mix IX/converter so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.5 Developer Solution III/HK Developer (lyophilized, 30 mg):

Reconstitute Developer Solution III/HK Developer in 220 µL of ddH_2O. Pipette up and down to dissolve completely. Aliquot developer solution/developer so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.6 HK Positive Control/Hexokinase (positive control) (lyophilized, 0.5 U):

Reconstitute HK Positive Control/Hexokinase in 160 µL of Assay Buffer LX/HK Assay Buffer. Aliquot positive control that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.7 Hexokinase Inhibitor/HK Inhibitor Control (lyophilized 40 µmole):

Reconstitute Hexokinase Inhibitor/HK Inhibitor Control in 100 µL of ddH_2O. Aliquot inhibitor so that you have enough volume to perform the desired number of assays. Store at - 20°C. Use within 2 months. Keep on ice while in use.

Immediately prior to use, dilute 1:5 in Assay Buffer: 20 µL Inhibitor Control + 80 µL Assay Buffer LX/HK Assay Buffer.

10. Sample Preparation

General sample information:

– Always prepare a fresh set of samples and controls for every use.

10.1 Screening Compounds:

10.1.1 Dissolve test compounds into proper solvent.

10.1.2 Dilute to 2X the desired test concentration with Assay Buffer LX/HK Assay Buffer before use.

Δ Note: We suggest using different concentrations of test compounds if effective concentration is unknown.

11. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.

Δ Note: preferred final solvent concentration should not be more than 5% by volume. If solvent exceeds 5%, include solvent control to test the effect of the solvent on enzyme activity.

11.1 Set up Reaction wells:

- Sample compound wells (S) = 50 µL test compounds.
- Inhibitor Control wells (IC): 50 µL diluted Inhibitor Control.
- Enzyme Control wells (EC) = 50 µL Assay Buffer LX/HK Assay Buffer.
- OPTIONAL: Solvent control (SC) = 50 µL solvent.

11.2 Prepare Hexokinase Enzyme Solution:

11.2.1 Dilute HK Positive Control/Hexokinase 1:10 with Assay Buffer LX/HK Assay Buffer. Mix well.

11.2.2 Add 5 µL diluted Hexokinase Enzyme Solution to each well.

11.2.3 Incubate for 5 minutes at 25°C.

Δ Note: Discard the diluted Hexokinase Enzyme Solution after use.

11.3 Hexokinase Substrate Solution Mix:

11.3.1 Prepare 45 µL of Substrate Solution Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix of the Reaction mix to ensure consistency.

Component	HK Substrate Mix (µL)
Assay Buffer LX/HK Assay Buffer	29
HK Substrate	10
HK Coenzyme	2
HK Converter	2
Developer Solution III/HK Developer	2

11.3.2 Add 45 µL of Hexokinase Substrate Mix into each well.
11.3.3 Mix well with gentle shaking.

11.4 Measurement:

11.4.1 Measure immediately absorbance (OD = 450 nm) on a microplate reader in kinetic mode for 5 - 30 minutes at 25°C.

12. Calculations

– Use only the linear rate for calculation.

12.1 Plot readings for each sample test compound (S), inhibitor control (IC) and enzyme control (EC).

12.2 Draw the line of the best fit to construct the curve (most plate reader software or Excel can do this step). Calculate the trend line equation (use the equation that provides the most accurate fit).

12.3 Choose two points (T1 and T2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD1 and OD2).

12.4 Calculate Slope (ΔOD/ΔT) for all samples (S), Enzyme Control (EC) and Inhibitor control (IC), if desired, as follows:

$$\Delta OD/\Delta T = (OD2 - ODU1)/ (T2 - T1)$$

12.5 Average the slope for each duplicate reading.

12.6 Calculate the % Relative Inhibitions as follows:

$$\%\ Relative\ Inhibition = \frac{Slope\ of\ EC - Slope\ of\ S}{Slope\ of\ EC}\ x\ 100$$

Δ Note: Irreversible inhibitors that inhibit the Hexokinase activity completely at the tested concentration will have ΔOD = 0 and thus % Relative Inhibition will be 100%.

Δ Note: If OD of SC < OD of EC = make a higher stock of test inhibitor, or dissolve the inhibitor in lower concentration of the solvent; or use a different solvent if possible.

If OD of S < OD of EC = treat as 100% inhibition and further dilute the test inhibitor and repeat the assay.

13. Typical Data

Data provided **for demonstration purposes only.**

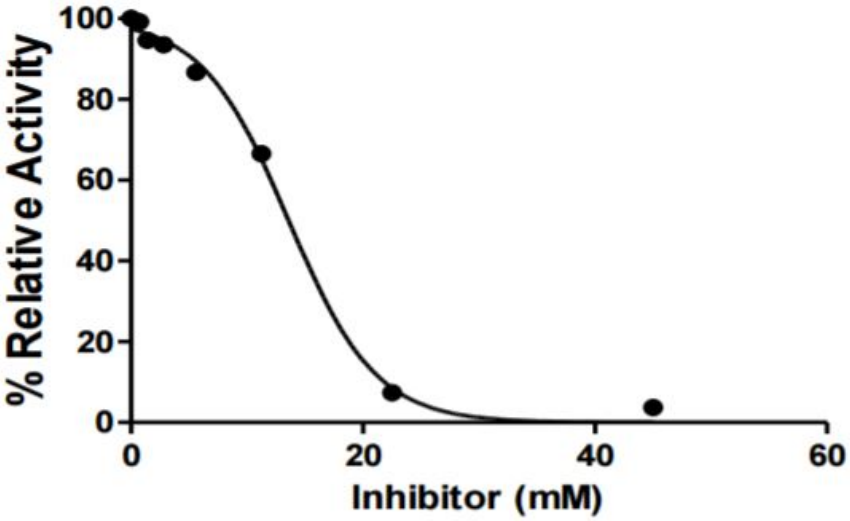


Figure 1. Typical inhibition curve of Hexokinase activity by Hexokinase Inhibitor (Bromopyruvic Acid). Assay was performed following the kit protocol.

14. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare reagents and aliquot; get equipment ready.
- Prepare test compounds in suitable solvents; dilute if appropriate.
- Set up Sample compound wells (S) (50 µL test compounds), Inhibitor Control wells (IC) (50 µL diluted 1:5 Inhibitor Control), Enzyme Control wells (EC) (50 µL Assay Buffer LX/HK Assay Buffer), Solvent control (SC) (50 µL solvent).
- Prepare Hexokinase Enzyme Solution (5 µL/well) by diluting HK Positive Control/Hexokinase 1:10 with Assay Buffer LX/HK Assay Buffer. Prepare a mix for all wells.
- Add 5 µL HK Enzyme Solution to each well. Incubate for 5 minutes at 25ºC.
- Prepare Hexokinase Substrate Mix (45 µL/well) master mix as follows:

Component	Hexokinase Substrate Mix (µL)
Assay Buffer LX/HK Assay Buffer	29
HK Substrate	10
HK Coenzyme	2
HK Converter	2
Developer Solution III/HK Developer	2

- Add 45 µL/Substrate mix to each well.
- Measure absorbance (OD = 450 nm) on a microplate reader in kinetic mode for 5 - 30 minutes at 25ºC.

15. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Assay with erratic readings	Pipetting errors	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
No fluorescence above background in inhibitor wells	Inhibitor concentration is too high	Reduce concentration of inhibitor and re-do assay
No inhibition seen in test compound wells	Inhibitor concentration is not high enough	Increase concentration of inhibitor and re-do assay
	Compound is not an inhibitor	Use another compound for your test

16. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)