Hexokinase Assay Kit (BA0248)

Product Datasheet | SKU BA0248

Description

A quantitative colorimetric kinetic assay for hexokinase activity. Hexokinase activity is measured through an NADPH-coupled reduction of the tetrazolium salt MTT to a coloured product with an absorption maximum at 565 nm.

Specifications

Detection Method	Colorimetric kinetic (OD565nm)
Detection Range	0.22 to 100 U/L
Sample Type	Microorganisms, plasma, serum, cultured cells, tissue and culture media
Species Reactivity	All
Assay Time	15 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	-20°C
Shelf Life	6 months after receipt
Shipping	Gel Pack

Kit Components

Component	Quantity	Storage
Assay Buffer	10 mL	-20°C
NADP/MTT	1 mL	-20°C
Substrate Mix	1 mL	-20°C
Enzyme A	120 µL	-20°C
Enzyme B	120 µL	-20°C
Calibrator	1 mL	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0248	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent should be quick and mixing brief but thorough; a multi-channel pipettor is recommended. Assays may be run at any desired temperature (e.g. 25°C or 37°C).
2. Sample preparation: serum and plasma are assayed directly. Tissue: rinse in phosphate buffered saline (pH 7.4) to remove blood, homogenise 50 mg tissue in ~200 µL buffer containing 50 mM potassium phosphate (pH 7.5), centrifuge at 10,000 × g for 15 min at 4°C and remove supernatant. Cell lysate: collect cells by centrifugation at 2,000 × g for 5 min at 4°C (use a cell scraper for adherent cells), homogenise or sonicate in cold buffer containing 50 mM potassium phosphate (pH 7.5), centrifuge at 10,000 × g for 15 min at 4°C and remove supernatant. Samples may be stored at -20 to -80°C for at least one month.

3. Reagent preparation: equilibrate reagents to the desired reaction temperature and briefly centrifuge tubes. Prepare enough Working Reagent (WR) for all wells by mixing, for each 96-well assay: 8 µL Substrate Mix, 8 µL NADP/MTT solution, 1 µL Enzyme A, 1 µL Enzyme B and 70 µL Assay Buffer.
4. Transfer 100 µL H2O (OD_H2O) and 100 µL Calibrator (OD_CAL) into wells of a clear flat-bottom 96-well plate.
5. Transfer 20 µL of each sample into separate wells, then add 80 µL WR to each sample well. Tap the plate briefly to mix.
6. Read OD565nm (OD0), and again after 15 min (OD15) on a plate reader.

Calculation

Subtract OD0 from OD15 for each sample to compute ΔOD_S. Hexokinase activity = (ΔOD_S / (OD_CAL – OD_H2O)) × (Reaction Vol / (t × Sample Vol)) × 273 × n (U/L), where 273 relates to the molar absorption coefficient of reduced MTT and light pathlength calculated from the calibrator, OD_CAL and OD_H2O are the OD565nm (OD0) values of the calibrator and water, t is the reaction time (15 min recommended), Reaction Vol and Sample Vol are 100 µL and 20 µL respectively, and n is the dilution factor. Unit definition: 1 U of hexokinase catalyses the conversion of 1 µmole of glucose to glucose-6-phosphate per min at pH 8.2. If activity exceeds 100 U/L, use a shorter reaction time or dilute the sample; for activity < 1 U/L, extend incubation to 2 hours.

Notes

- Normal precautions for laboratory reagents should be exercised while using the reagents.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.