Sorbitol Dehydrogenase Assay Kit (Colorimetric)

Catalog Number KA4567

100 assays

Version: 01

Intended for research use only

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Introduction

Intended Use

Applications:

- ✓ SDH activity determination in biological samples (e.g. plasma, serum, urine, tissue and culture media.)

Features:

- ✓ Fast and sensitive. Linear detection range (20 µL sample): 0.1 to 125 U/L for 12 min reaction.
- ✓ Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Background

SORBITOL DEHYDROGENASE (SDH) is an enzyme that catalyzes the interconversion of sorbitol and fructose. Elevated blood serum SDH levels indicate liver damage; thus, SDH plays an important role in the diagnosis of liver disease, especially in combination with aminotransferases. SDH levels are also measured to evaluate diabetic complications such as proliferative diabetic retinopathy.

This non-radioactive, colorimetric SDH assay is based on the reduction of the tetrazolium salt MTT in a NADH-coupled enzymatic reaction to a reduced form of MTT which exhibits an absorption maximum at 565 nm. The increase in absorbance at 565 nm is directly proportional to the enzyme activity.

General Information

Materials Supplied

List of component

Component	Amount
Assay Buffer	10 mL
Diaphorase	120 µL
Substrate	250 µL
Calibrator	1.5 mL
NAD/MTT Solution	1 mL

Storage Instruction

Store all components at -20°C upon receiving.

Materials Required but Not Supplied

1. Pipetting devices and accessories (e.g. multi-channel pipettor)
2. Clear flat-bottom 96-well plates (e.g. Corning Costar)
3. Centrifuge tubes
4. Plate reader

Precautions for Use

Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents.

Assay Protocol

Reagent Preparation

Equilibrate reagents to desired reaction temperature (37°C is recommended). Briefly centrifuge tubes before use.

Sample Preparation

Serum and plasma can be assayed directly.

- ✓ Tissue: prior to dissection, rinse tissue in phosphate buffered saline (PBS, pH 7.4) to remove blood. Homogenize tissue (50 mg) in 200 µL cold PBS buffer. Centrifuge at 14,000 x g for 5 min at 4°C. Remove supernatant for assay.
- ✓ Cell Lysate: collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold PBS buffer. Centrifuge at 14,000 x g for 5 min at 4°C. Remove supernatant for assay.
- ✓ All samples can be stored at –20 to –80°C for at least one month.

Assay Procedure

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at any desired temperature (e.g. 25°C or 37°C).

1. Transfer 100 µL dH 2 O (OD H2O) and 100 µL Calibrator (OD CAL) solution into wells of a clear flat bottom 96-well plate.
2. Transfer 20 µL dH2O into one well, this will be the blank. Transfer 20 µL of each sample into separate wells.
3. Prepare enough Working Reagent (WR) for all reaction wells by mixing, for each 96-well assay, 2 µL Substrate, 8 µL NAD/MTT Solution, 1 µL Diaphorase and 75 µL Assay Buffer.
4. Add 80 µL WR to all sample and blank wells. Tap plate briefly to mix.
5. Incubate at desired temperature; read OD 565nm at time 3 min (OD 3) and time 15 min (OD 15) on a plate reader.

Data Analysis

Calculation of Results

Subtract the OD 3 from OD 15 for each sample well to compute the ΔOD S values, do the same for the blank to compute ΔOD B. SDH activity can then be calculated as follows:

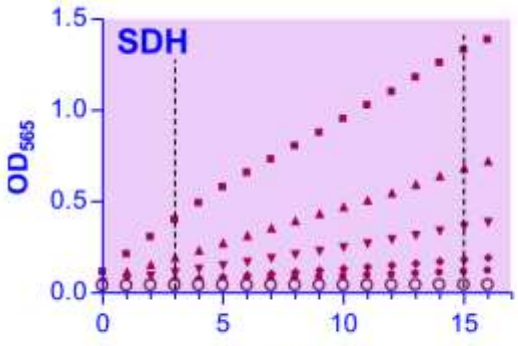
$$\text{SDH Activity} = \frac{\Delta OD\ S - \Delta OD\ B}{\varepsilon_{mtt} \bullet l} \times \frac{\text{Reaction Vol (µL)}}{t\,(min) \bullet \text{Sample Vol (µL)}} \times n$$

$$= \frac{273}{t\,(min)} \times \frac{\Delta OD\ S\ - \Delta OD\ B}{OD\ CAL - OD\ H20} \times n\ (U/L)$$

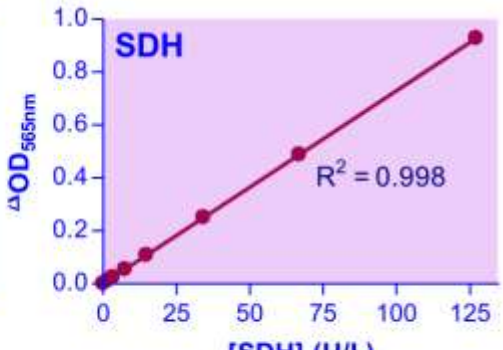
where ε mtt is the molar absorption coefficient of reduced MTT. l is the light pathlength which is calculated from the calibrator. OD CAL and OD H20 are OD 565nm (OD 3) values of the Calibrator and water. t is the difference in time between readings (15 min minus 3 min = 12 min is the recommended time). Reaction Vol and Sample Vol are 100 µL and 20 µL, respectively. n is the dilution factor.

Unit definition: 1 Unit (U) of SDH will catalyze the conversion of 1 µmole of D-sorbitol to fructose per min at pH 8.2.

Note: If sample SDH activity exceeds 125 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with SDH activity < 1 U/L, the reaction time can be extended to 2 hours. We recommend running kinetics and choosing two time points in which the activity remains linear.



Raw Kinetic Data (37˚C)



SDH Activity (15 min, 37 °C)

Resources

References

1. Uzozie, A et al (2014). Sorbitol Dehydrogenase overexpression and other aspects of dysregulated protein expression in human precancerous colorectal neoplasms: a quantitative proteomics study. Mol Cell Proteomics. 13(5):1198-1218.
2. Linstad, RI at al (2013). Inhibition of Sorbitol Dehydrogenase by nucleosides and nucleotides. Biochem Biophys Res Commun. 435(2): 202-208.
3. Aguayo, MF et al (2013). Sorbitol dehyodrogenase is a cytosolic protein required for sorbitol metabolism in Arabidopsis thalania. Plant Sci. 205-206: 63-75.