Technical Manual

ChromaDazzle Sorbitol Dehydrogenase Activity Assay Kit

Catalogue Code: BA0056

Pack Size: 100 assays

Research Use Only

DESCRIPTION

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.

The Assay Genie non-radioactive, colorimetric ChromaDazzle Sorbitol Dehydrogenase (SDH) Activity Assay Kit 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.

KEY 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.

APPLICATIONS

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

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

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

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

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).

Sample Preparation: Serum and plasma are 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.

Reagent Preparation: equilibrate reagents to desired reaction temperature (37°C is recommended). Briefly centrifuge tubes before use.

Assay

1. Transfer 100 µL dH_2O (OD_{H2O}) and 100 µL Calibrator (OD_{CAL}) solution into wells of a clear flat bottom 96-well plate.
2. Transfer 20 µL dH_2O 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.

Add 80 µL WR to all sample and blank wells. Tap plate briefly to mix.

4. Incubate at desired temperature; read OD_{565nm} at time 3 min (OD_3) and time 15 min (OD_{15}) on a plate reader.

CALCULATION

Subtract the OD_3 from OD_{15} for each sample well to compute the DOD_S values, do the same for the blank to compute DOD_B. SDH activity can then be calculated as follows:

$$\text{SDH Activity} = \frac{\Delta OD_S - \Delta OD_B}{\varepsilon_{mtt} \cdot l} \times \frac{\text{Reaction Vol (µL)}}{t\,\text{(min)} \cdot \text{Sample Vol (µL)}} \times n$$

$$= \frac{273}{t\,\text{(min)}} \times \frac{\Delta OD_S - \Delta OD_B}{OD_{CAL} - OD_{H20}} \times n \quad (U/L)$$

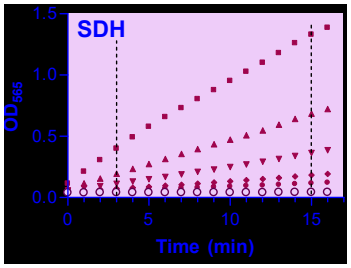
where ε_{mtt} is the molar absorption coefficient of reduced MTT. l is the light pathlength which is calculated from the calibrator. OD_{CAL} and OD_{H20} are OD_{565nm} (OD_3) values of the Calibrator and water. t is the difference in time between readings (15 min minus 3 min = 12 min is the recommended 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.

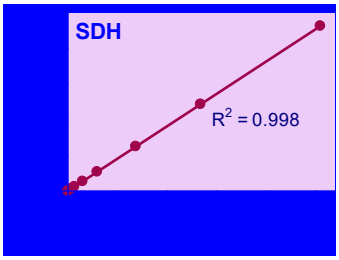
Note: If sample SDH activity exceeds 125 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with SDH activity < 1 U/L, the reaction time can be extended to 2 hours. We recommend running kinetics and choosing two time points in which the activity remains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

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. Corning Costar), centrifuge tubes and plate reader.



Raw Kinetic Data (37˚C)



SDH Activity (15 min, 37 ˚C)

LITERATURE

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.

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