Sorbitol Dehydrogenase Activity Assay Kit (Colorimetric)

Sorbitol Dehydrogenase Activity Assay Kit (Colorimetric) can be used to measure Sorbitol Dehydrogenase activity in serum, plasma, urine, tissue and cell culture supernatants.

Catalog number: ARG82192

Package: 100 tests

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Sorbitol dehydrogenase (or SDH) is a cytosolic enzyme. In humans this protein is encoded by the SORD gene.

Sorbitol dehydrogenase is an enzyme in carbohydrate metabolism converting sorbitol, the sugar alcohol form of glucose, into fructose. Together with aldose reductase, it provides a way for the body to produce fructose from glucose without using ATP. Sorbitol dehydrogenase uses NAD+ as a cofactor; its reaction is sorbitol + $NAD^+ \rightarrow$ fructose + NADH + H^+. A zinc ion is also involved in catalysis. Organs that use it most frequently include the liver and seminal vesicle; it is found in various organisms from bacteria to humans. A secondary use is the metabolism of dietary sorbitol, though sorbitol is known not to be absorbed as well in the intestine as its related compounds glucose and fructose, and is usually found in quite small amounts in the diet (except when used as an artificial sweetener). [Provide by Wikipedia: Sorbitol dehydrogenase]

PRINCIPLE OF THE ASSAY

This Sorbitol dehydrogenase Activity Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of Sorbitol dehydrogenase (SDH) present in plasma, serum, urine, tissue and cell culture supernatant. This 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 O.D. 565 nm. The increase in absorbance at O.D. 565 nm is directly proportional to the enzyme activity.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Assay Buffer	10 mL	-20°C
Diaphorase	120 µL	-20°C
Substrate	250 µL	-20°C
NAD/MTT Solution	1 mL	-20°C
Standard	1.5 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 565 nm
- Clear flat-bottom 96 well microplate
- Centrifuge and centrifuge tubes
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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).
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collect the serum and assay directly.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collect the plasma layer and assay directly.

Urine: assayed directly. If particulates are present, centrifuge at 2,000 x g for 5 minutes at 4°C. Collect the supernatant and assay directly.

Cell lysate: Collect cell by centrifugation at 2,000 x g for 5 minutes at 4°C. Homogenize or sonicate cells in an appropriate volume of cold PBS. Centrifuge at 14,000 x g for 5 minutes at 4°C. Collect the supernatant and assay directly.

Tissue lysate: prior to dissection, rinse tissue in PBS (pH 7.4) to remove blood. Homogenize tissue (50 mg) in 200 µL of cold PBS. Centrifuge at 14,000 x g for 5 minutes at 4°C. Collect the supernatant and assay directly.

Note:

- For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman.
- All samples can be stored at -20 to -80°C for at least one month.

REAGENT PREPARATION

- **Working Reagent:** for each reaction, mixing 75 µL of Assay Buffer, 8 µL of NAD/MTT Solution, 2 µL of Substrate and 1 µL of Diaphorase. Fresh reconstitution is recommended.

ASSAY PROCEDURE

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

	Standard well	H_2O well	Sample well	Blank well
Standard	100 µL			
Distilled water		100 µL		20 µL
Each Sample			20 µL	
Working Reagent			80 µL	80 µL
Tap plate to mix well. Incubate at **desired temperature (37°C** is recommended).				
Read the **O.D. 565 nm** at **3 minutes (OD_3)** and **15 minutes (OD_{15}).**				

Note: 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.

CALCULATION OF RESULTS

1. Subtract the OD_3 from OD_{15} for each sample to compute the ΔOD_S values, do the same for blank to compute ΔOD_B.

   SDH activity can then be calculated as follows:

   SDH Activity (U/L)

   $= [(\Delta OD_S - \Delta OD_B) / (\varepsilon_{mtt} \times l)] \times [\text{Reaction Vol} / (t \times \text{Sample Vol})] \times n$

   $= (273 / t) \times [(\Delta OD_S - \Delta OD_B) / (OD_{STANDARD} - OD_{H2O})] \times n$

   Note:

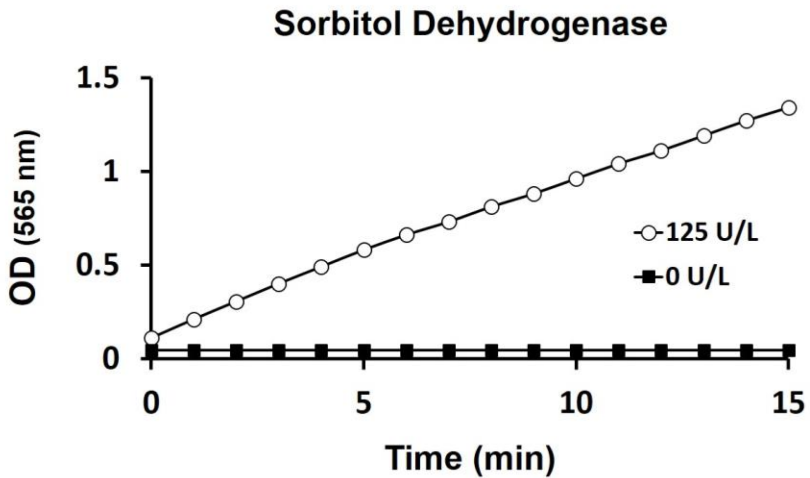
   - ε_{mtt}: the molar absorption coefficient of reduced MTT.
   - *l*: the light path length which is calculated from the calibrator.
   - $OD_{STANDARD}$ and OD_{H2O}: the O.D. 565 nm (OD_3) value of Standard and H_2O wells.
   - t: the difference in time between readings (15 - 3 = 12 minutes is the recommended)
   - Reaction Vol and Sample Vol: 100 µL and 20 µL, respectively.
   - n: the dilution factor if the sample needed to be diluted.

2. 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.

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

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Sorbitol Dehydrogenase Activity Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.1 U/L