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Product Information

Sorbitol Dehydrogenase Kit

Catalog Number **MAK317**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product 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.

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

This assay is based on a kinetic reaction. Sorbitol dehydrogenase (SDH) activity can be determined in a number of biological samples (e.g., plasma, serum, urine, tissue, and culture media).

Fast and sensitive – Linear detection range (20 µL sample): 0.1–125 Units/L for 12 minute 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.

Components
The kit is sufficient for 100 assays in 96 well plates.

Assay Buffer Catalog Number MAK317A	10 mL
Substrate Catalog Number MAK317B	250 µL
NAD/MTT Solution Catalog Number MAK317C	1 mL
Diaphorase Catalog Number MAK317D	120 µL
Calibrator Catalog Number MAK317E	1.5 mL

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate or cuvettes – It is recommended to use clear plates for colorimetric assays
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Allow all reagents to come to room temperature before use. Equilibrate reagents to desired reaction temperature (37 °C is recommended).

Storage/Stability
The kit is shipped at room temperature. Storage at –20 °C is recommended.

Procedure

All samples and standards should be run in duplicate. Use ultrapure water for the preparation of samples and standards.

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 of cold PBS buffer. Centrifuge at 14,000 × *g* for 5 minutes at 4 °C. Remove supernatant for assay.

Cell Lysate – collect cells by centrifugation at 2,000 × *g* for 5 minutes 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 × *g* for 5 minutes at 4 °C. Remove supernatant for assay. All samples can be stored at –20 to –80 °C for at least one month.

Assay Reaction

1. Transfer 100 µL of ultrapure water (ODH2O) and 100 µL of Calibrator (ODCAL) solution into wells of a clear flat bottom 96-well plate.
2. Transfer 20 µL of water 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 of Substrate, 8 µL of NAD/MTT Solution, 1 µL of Diaphorase and 75 µL of Assay Buffer. Add 80 µL of WR to all sample and blank wells. Tap plate briefly to mix.
   Note: To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multichannel pipettor is recommended.
4. Incubate at desired temperature; read OD565nm at time 3 minutes (OD3) and time 15 minutes (OD15) on a plate reader. Assays can be executed at any desired temperature (e.g. 25 °C or 37 °C).

Results

Calculations

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:

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

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

Where:

ε_{mtt} = the molar absorption coefficient of reduced MTT.

l = the light pathlength which is calculated from the calibrator.

OD_{CAL} and OD_{H20} = OD_{565nm} (OD_3) values of the Calibrator and water.

t = the difference in time between readings (15 min minus 3 min = 12 min is the recommended time).

Reaction Vol and Sample Vol = 100 µL and 20 µL, respectively.

n = the dilution factor.

Unit definition: 1 Unit (U) of SDH will catalyze the conversion of 1 µmole of D-sorbitol to fructose per minute 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. Running kinetics is recommended and choosing two time points between which the activity remains linear.

References

1. Uzozie, A. et al., 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 (2014).
2. Linstad, R.I. et al., Inhibition of Sorbitol Dehydrogenase by nucleosides and nucleotides. Biochem. Biophys. Res. Commun., **435**(2), 202-208 (2013).
3. Aguayo, M.F. et al., Sorbitol dehyodrogenase is a cytosolic protein required for sorbitol metabolism in *Arabidopsis thalania*. Plant Sci., **205-206**, 63-75 (2013).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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