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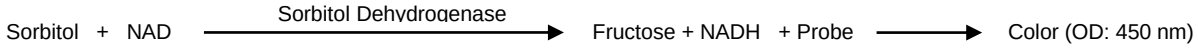
Sorbitol Dehydrogenase Activity Assay Kit (Colorimetric)

rev 11/19

(Catalog # K935-100; 100 assays; Store at -20°C)

I. Introduction:

Sorbitol Dehydrogenase (SDH), also referred to as L-iditol dehydrogenase (EC 1.1.1.14) participates in the polyol pathway, also known as the sorbitol-aldose pathway. This pathway is divided into two steps: first, glucose is reduced to sorbitol via the catalytic action of aldose reductase (AR), while the second step is catalyzed by sorbitol dehydrogenase, which utilizes sorbitol as the substrate and subsequently produces fructose. Sorbitol is not a cell membrane permeable molecule whereas fructose can be further metabolized inside the cells. Recent studies found that the deficiency of Sorbitol Dehydrogenase causes the intracellular accumulation of sorbitol, which leads complications in diabetes. The enzyme is predominately expressed in the liver cells and is only released into the bloodstream following acute liver damage. Therefore, the presence of significant SDH activity in serum is a potential indicator of liver injury or disease. **BioVision's SDH activity assay kit** provides a quick and easy way for monitoring SDH activity in various samples. In this Assay, Sorbitol Dehydrogenase utilizes a provided substrate while reducing NAD^+ to form NADH. NADH reacts with the developer, leading to the formation of a chromophore with strong absorbance at OD 450 nm. The assay is simple, sensitive and can detect Sorbitol Dehydrogenase Activity less than 50 µU in variety of samples.



II. Application:

- Measurement of Sorbitol Dehydrogenase Activity in various tissues/cells.
- Analysis of polyol-pathway.
- Studies of chemically-induced hepatotoxicity and acute liver injury.
- Freshly obtained serum from animals with suspected liver damage

III. Sample Type:

- Animal tissues: Liver, Kidney etc.
- Cell culture: HeLa, Jurkat etc.

IV. Kit Contents:

Components	K935-100	Cap Code	Part Number
SDH Assay Buffer	25 ml	WM	K935-100-1
SDH Substrate	200 µl	Blue	K935-100-2
SDH Developer	1 vial	Red	K935-100-3
NADH Standard	1 vial	Yellow	K935-100-4
SDH Positive Control	1 vial	Purple	K935-100-5

V. User Supplied Reagents and Equipment:

- 96-well plate with flat clear bottom
- Multi-well spectrophotometer (ELISA reader)
- Ammonium Sulfate Solution (Saturated, 4.3 M), BioVision Cat# 7096

VI. Storage Conditions and Reagent Preparation:

Store kit at –20°C, protected from light. Warm all buffers to room temperature (RT) before use. Briefly centrifuge all small vials prior to opening.

- **SDH Developer:** Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **NADH Standard:** Reconstitute with 500 µl Assay buffer to generate 1 mM NADH Standard solution. Store at –20°C. Use within two months. Keep on ice while in use.
- **SDH Positive Control:** Reconstitute with 100 µl of dH_2O and mix thoroughly to prepare SDH Positive Control stock. Divide into aliquots and store at –20°C.

VII. Sorbitol Dehydrogenase Assay Protocol:

1. **Sample Preparation:** For whole cells or tissue lysate, rapidly homogenize tissue (10 mg) or cells (2 x 10^6) with 200 µl ice cold SDH Assay Buffer, and place on ice for 10 minutes. Centrifuge at 10,000 X g for 5 min and collect the supernatant. Use the ammonium sulfate precipitation method to remove interfering small molecules: Aliquot the tissue samples (100 µl) to a clean centrifuge tube, add saturated 4.32 M ammonium sulfate (BioVision Cat. # 7096) to 65% saturation (1 volume of sample + 2 volumes of 4.32M ammonium sulfate) and place on ice for 30 mins. Spin down samples at 10,000 x *g* at 4°C for 10 min, discard the supernatant, and resuspend the pellet back to the original volume.
2. **SDH Assay:** Add 2-50 µl of each sample into a 96 well clear plate. Adjust the final volume to 50 µl with SDH Assay Buffer. For SDH Positive Control, dilute 2 µl of SDH Positive Control stock with 18 µl SDH Assay Buffer, mix well. Add 2-20 µl of the diluted Positive Control and adjust final volume of each well to 50 µl with SDH assay buffer. Aliquot and store the rest of SDH stock at -20°C.

   Notes:

   a. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

b. For samples exhibiting elevated background, prepare parallel sample wells as sample background controls.

3. **NADH Standard Curve:** Add 0, 2, 4, 6, 8 and 10 µl of 1 mM NADH Standard into a series of wells in duplicate in 96 well plate to generate 0, 2, 4, 6, 8 and 10 nmol/well of NADH Standard. Adjust volume to 50 µl/well with Assay Buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	Background Control Mix
SDH Assay Buffer	46 µl	48 µl
SDH Developer	2 µl	2 µl
SDH Substrate	2 µl	----

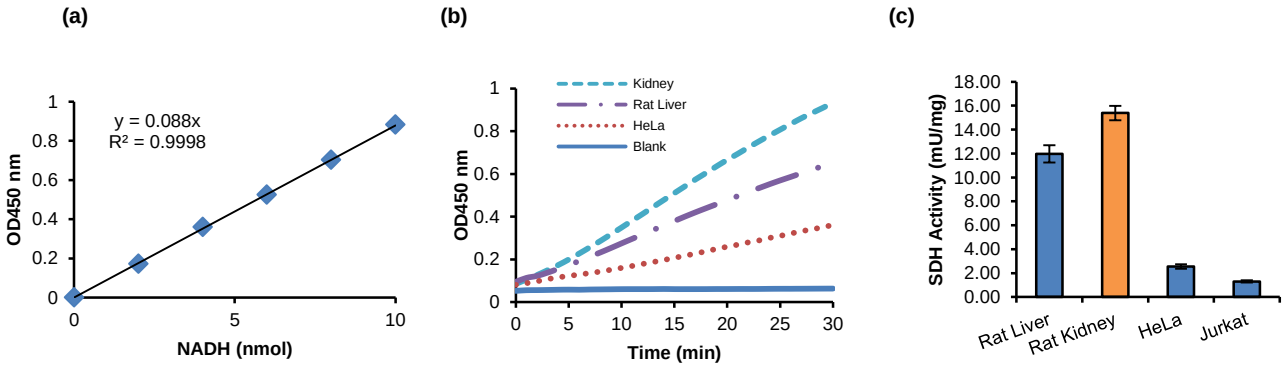
Add 50 µl of the Reaction Mix to each well containing the Standard, Positive Control and test samples and 50 µl of Background Control mix to each well containing Sample Background Control. Mix well.

4. **Measurement:** Measure absorbance immediately at 450 nm in kinetic mode for 5-60 min at 37ºC.
Note: Incubation time depends on the Sorbitol Dehydrogenase activity in the samples. We recommend measuring the OD in a kinetic mode, and choose two time points (t_1 & t_2) in the linear range to calculate the SDH activity of the samples. The NADH Standard Curve can be read in Endpoint mode (i.e., at the end of incubation time).
5. **Calculation:** Subtract the 0 Standard reading from all Standard readings. Plot the NADH Standard Curve. Correct Sample Background by subtracting the value derived from the Sample Background Controls from their respective sample readings. Calculate the signal generated by SDH activity of the test sample: $\Delta OD = A_2 - A_1$. Apply the ΔOD to the NADH Standard Curve to get B nmol of NADH generated by Sorbitol Dehydrogenase during the reaction time ($\Delta T = t_2 - t_1$).

Sample Sorbitol Dehydrogenase Activity = B/(ΔT X V) x Dilution Factor = nmol/min/ml/ = mU/ml

Where: **B =** the NADH amount from the Standard Curve (nmol).
ΔT = the reaction time (min).
V = the sample volume added into the reaction well (ml).

Unit Definition: One unit of Sorbitol Dehydrogenase is the amount of enzyme that will generate 1.0 µmol of NADH per min at pH 9 at 37ºC.



Figures: (a) NADH Standard Curve. (b) Kinetic measurement of Sorbitol Dehydrogenase activity from various samples. (C) Relative SDH Activity was calculated in lysates (µg protein) prepared from Rat Liver (8 µg), Rat Kidney (10 µg), HeLa (42 µg) and Jurkat (73 µg). Assays were performed following kit protocol.

VIII. Related Products:

Glucose and Sucrose Assay Kit (K616)
Glucose Uptake Colorimetric Assay Kit (K676)
Glucose Uptake Fluorometric Assay Kit (K666)
NAD/NADH Quantification Kit (K337)
PicoProbe™ Glucose-6-Phosphate Assay Kit (K687)
Phosphoglucomutase Assay Kit (K774)
D-Sorbitol Colorimetric Assay Kit (K631)
Glucose-6-Phosphate Dehydrogenase Assay Kit (K757)
Fructose Assay kit (K619)
Hexokinase Assay Kit (K789)
Glucose Dehydrogenase Activity Assay Kit (K679)
Free Glycerol Colorimetric Assay Kit (K634)

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