VetSpec ™ SORBITOL DEHYDROGENASE (SDH))
FOR MANUAL /AUTOMATED ASSAYS
C432-0B, C434-0A, C434-0C

Intended Use

For **In Vitro Diagnostic** use in the automated, quantitative determination of SDH in serum or plasma.

Clinical Significance (1-4)

Measurement of SDH activity is of considerable clinical value as an effective indicator of acute hepatic anoxia. Studies by Gertch and others have supported this premise by reporting sharp increases in serum SDH activity in cases of extensive liver damage such as in acute hepatitis.

Method Principle (1)

Several procedures have been reported in the literature for measuring the enzyme, L-Iditol Dehydrogenase or Sorbitol Dehydrogenase (SDH, EC 1.1.1.14) in serum. Catachem's procedure is based on the enzymatic procedure described by Clive I. Rose and Arthur R. Henderson. In this procedure SDH catalyzes the revesible oxidation-reduction reaction between sorbitol and fructose with concomitant oxidation of NADH to NAD^+. The decrease in absorbance is monitored at 340nm. The delta absorbance produced is directly proportional to the concentration of SDH activity in the serum sample. The reaction scheme below illustrates the reaction that takes place in this SDH procedure.

REAGENTS

SDH Sample Diluent Reagent (R1)
Each liter contains:

Fructose	4.00 mol
Stabilizer and non-reactive ingredients	

SDH Activator Reagent (R2)
Each liter contains:

Buffer	
NADH	0.20 mmol
Stabilizer and non-reactive ingredients.	

Precautions

Handle these reagents using good laboratory practice. **DO NOT PIPETTE REAGENT BY MOUTH.** Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Clean spills immediately.

Reagent Storage and Stability

Store the SDH Sample Diluent (R1) and the SDH Activator Reagent (R2) at 2-8°C. When stored unopened as directed, these reagents are stable until the expiration date stated on the label.

Reagent Preparation

Both reagents are ready to use. Once opened, each reagent, R1 and R2, is stable for 60 days when stored at 2-8°C and capped tightly when not in use.

Indications of Reagent Deterioration

- Turbidity
- Quality control values out of assigned ranges.

If these reagent characteristics are observed contact your technical representative.

Specimen Collection and Stability

Clear unhemolyzed sera are the specimens of choice. Serum should be separated immediately from the clot and analyzed promptly or stored at 2-8°C. SDH serum is stable 7 days refrigerated at 2-8°C and for several months frozen at -20°C. (1)

Procedure

These instructions are outlined for performing the SDH assays using a manual procedure.

Quality Control

To monitor the quality performance of the procedure used, Catachem's SDH Control Level I and Control Level II, or other appropriate controls with assigned SDH values should be included in the assay procedure each time the assay is performed. These materials are available separately from this kit.

Interfering Substances

A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young et al (5).

Procedure Limitations

Samples with SDH values greater than 50 U/L should be diluted 1:2 with physiological saline and reassayed. Multiply results obtained by 2 to adjust for the sample dilution.

Procedure

Important: Read the entire procedure instructions before proceeding with the assay.

Materials Provided

SDH Reagents (R1 and R2)

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Materials Required (But Not Provided)

Spectrophotometer

Cuvettes	1 cm light path
Timer	to time incubation time
Pipette	0.5 ml and 0.1 ml for reagents
Pipette	0.2 ml for sample
Cylinder	25 ml for reagent.

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Analytical Parameters

Wavelength	340nm
Temperature	37˚C
Pathlength	1 cm
Reaction Mode	Rate: zero order
Reaction Time:	5 minutes
Reagent Volume(R1):	0.50 ml
Reagent Volume(R2):	0.10 ml
Sample Volume:	0.05 ml
Total Volume:	0.65 ml
Sample-to-reagent ratio	1:13

Assay Procedure

1. Set spectrophotometer wavelength at 340nm and zero the instrument with the cuvette containing water.
2. Pipette 0.50 ml of Reagent (R1) into each of two cuvettes marked "Sample (s)" and "Control (s)".
3. Pipette 0.10 ml of Working Reagent (R2) into each of two cuvettes marked "Sample" and "Control".
4. Pipette 0.05 ml of control or sample into their respective cuvettes. Mix both cuvettes well.
5. Incubate both cuvettes for exactly 5 minutes at 37˚C and note the delta OD change. (or continuously monitor the change in absorbance for at least 5 minutes.)
6. Read the "Control" and "Sample" absorbencies.
7. Calculate the SDH concentration (U/L) in the sample(s), as shown in calculations and results.

Results and Calculations

$$\text{SDH activity u/L} = \frac{\Delta \text{ OD}}{\text{min}} \times \frac{\text{TV ml} \times 1000}{6.22 \times \text{L} \times \text{SV ml}}$$

Where:

Δ OD/min	= change in absorbance/minute
TV ml	= total volume in cuvette
SV ml	= volume of sample being assayed
6.22	= mmol coefficient of NADH at 340nm
L	= cuvette path length in cm.
1000	= converts U/ml to U/L

Example: ΔOD/min = 0.01

$$\text{SDH U/L} = \frac{0.01 \times 0.65 \times 1000}{6.22 \times 1.0 \times 0.05} = 20.1 \text{ U/L}$$

Method Performance Characteristics

Sensitivity: Using a pathlength of 1 cm, a Δ-absorbance of 0.0005-0.001 per U/L should be obtained.

Linearity: This procedure is linear over the range of 0-50 U/L.

References:

1. Rose CI, Henderson AR Clin Chem 21, 1619 (1975).
2. Asada M, Galambos JT. Sorbitol Dehydrogenase and hepatocellular injury. An experimental and clinicalstudy. Gastroenterology 44, 578 (1963).
3. Gerlach U. Zur Klinichen bedeutun der Aktivitatsmessung von Sorbidehydrogenase in Menslichen Blutserum. Klin Worchenschr 37, 93 (1959).
4. Blakley RL. The metabolism and antikitogenic effects of sorbitol dehydrogenase. Biochem J. 49, 257 (1951).
5. Young DS, Pestamer LC, Giberman V. Clin Chem 25, No. 5 (1975)

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