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. The Catachem procedure is based on the enzymatic procedure described by Clive I. Rose and Arthur R. Henderson. In this procedure SDH catalyzes the reversible 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.

$$\text{D- FRUCTOSE} + \text{NADH} \xrightarrow{\text{SDH}} \text{D-SORBITOL} + NAD^+$$

REAGENTS
SDH Sample Diluent Reagent (R1)
Each liter contains:

Buffer	
Fructose	4.00 mol
Stabilizer and nonreactive ingredients	

SDH Activator Working Reagent (R2)
Each liter contains:

NADH	2.0 mmol
Stabilizer and nonreactive 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
When stored at 2-8°C Catachem SDH Sample Diluent (R1) is stable until expiration date stated on the label. The unopened Catachem SDH Activator Reagent (R2) Powder and Diluent are stable until expiration date stated on the label when stored at 2-8°C.

Reagent Preparation
The Catachem SDH Sample Diluent Reagent (R1) is ready for use as is and is stable for at least 60 days after opening. The activator Reagent (R2), once prepared, is stable for at least 60 days. Both reagents should be stored capped when not in use at 2-8C.

Reagent Indications of Deterioration
- Turbidity
- Quality control values out of assigned ranges

If these reagent characteristics are observed call Catachem Technical service.

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 in 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 assay using a manual procedure. If running on an automated analyzer contact Catachem technical service for application assistance.

Materials Required but Not Provided
- Instrument
- Catachem SDH Quality Control material and Calibrator with assigned SDH values

Calibration
Catachem SDH Calibrator which contains a known SDH value is recommended.

Quality Control
To monitor the quality performance of the procedure used, Catachem SDH Control Level I and SDH Control Level II with assigned SDH values should be included in the assay procedure each time the assay is run.

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 100U/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 Required

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

Material Provided
SDH Reagents: Sample Diluent (R1), Activator (R2) Diluent and Activator (R2) Powder.

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. Prepare the SDH R2 working reagent, combine C432-24 and C432-25.
2. Set the spectrophotometer wavelength at 340 nm and zero the instrument with the cuvette containing water.
3. Pipette 0.50 ml of Reagent (R1) into each of two cuvettes marked “Sample (s)” and “Control (s)”.
4. Pipette 0.10 ml of Working Reagent (R2) into these same cuvettes marked “Sample” and “Control”. Bring cuvettes to 37^0 C.
5. Pipette 0.05 ml of control or sample into their respective cuvettes. Mix both cuvettes well.
6. Incubate both cuvettes for exactly 5 minutes at 37˚C after sample addition. Note the starting absorbance and final absorbance for both cuvettes.
7. Calculate the SDH concentration (U/L) in the sample(s) as shown in results and calculations.

Results and Calculations

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

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 0-100 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)

REV: 20240212SD

353 Christian Street Suite 2 ● Oxford, Connecticut ● 06478 USA ● Phone (203) 262-0330 ● Fax (203) 262-9836 ● email: catachem@catacheminc.com