**D-SORBITOL / XYLITOL (Colorimetric Method)**

### Intended use

Colorimetric Method For The Determination Of D-Sorbitol And Xylitol In Foodstuffs Such As Bakery Goods, Chocolate, Diabetic And Dietetic Food, Fruit Products, Soft Drinks, Sweets And Candies, As Well As In Pharmaceuticals, Cosmetics, Paper And Cardboard And In Biological Samples.

### General

D-Sorbitol, a sugar alcohol, the reduction product of D-fructose, occurs extensively in fruits, e.g. in apples, cherries, pears, plums, but it is not or only in traces contained in grapes, grape juice and wine. D-Sorbitol is used in the food industry as a moisturizing agent and as a sugar substitute for diabetic products as, in contrast to D-glucose, insulin is not necessary for metabolism. Xylitol is a sugar alcohol that occurs frequently in fruits, vegetables and mushrooms. It is used in liver infusion therapy.

**Test Principle:**

D-Sorbitol and xylitol are oxidized by nicotinamide-adenine dinucleotide (NAD) to D-fructose or D-xylulose, respectively, in the presence of the enzyme sorbitol dehydrogenase (SDH, also called polyol dehydrogenase) with the formation of reduced nicotinamide-adenine dinucleotide (NADH) (1a, 1b).

\[
\begin{align*}
(1a) & \quad \text{D-Sorbitol} + \text{NAD}^+ \xrightarrow{\text{SDH}} \text{D-Fructose} + \text{NADH} + \text{H}^+ \\
(1b) & \quad \text{Xylitol} + \text{NAD}^+ \xrightarrow{\text{SDH}} \text{D-Xylulose} + \text{NADH} + \text{H}^+
\end{align*}
\]

Under the assay conditions, the equilibrium of the reactions (1a, 1b) lies on the side of NAD and D-sorbitol or xylitol, respectively. However, they are favourably displaced as the formed NADH is removed in a subsequent reaction in which NADH reduces iodonitrotetrazolium chloride (INT) to a formazan in the presence of diaphorase (2).

\[
\begin{align*}
(2) & \quad \text{NADH} + \text{INT} + \text{H}^+ \xrightarrow{\text{diaphorase}} \text{NAD}^+ + \text{formazan}
\end{align*}
\]

The absorbance of the formazan is measured at its maximum at 492 nm.

### Materials Provided:

- 1 x Potassium phosphate/triethanolamine buffer solution, pH approx. 8.6; (25 ml) consisting of: Triton X-100 (trademark of Rohm & Haas, Philadelphia, USA).
- 3 x Lyophilizate; (35 mg); consisting of: diaphorase (approx. 4 U); NAD (approx. 28 mg).
- 1 x Iodonitrotetrazolium Chloride Solution, (approx. 2.5 ml).
- 3 x Lyophilizate SDH, (approx. 25 U, each).
- 1 x D-Sorbitol Assay Control Solution, for assay control purposes (measurement of the assay control solution is not necessary for calculating the results.) Use the assay control solution undiluted.

### Kit Specifications:

**Detection Limit:**
- 0.2 mg/l.

**Specificity:**
- Besides D-sorbitol and xylitol, sorbitol dehydrogenase also oxidizes other polyols, such as iditol, allitol, ribitol, although with reduced velocity. Other polyols such as mannitol, arabitol, and dulcitol do not react. Glycerol is not oxidized under the assay conditions.

**Linearity:**
- Linearity of the determination exists from 0.4 µg D-sorbitol or xylitol/assay (0.2 mg D-sorbitol or xylitol/l sample solution; \(v = 2.000\) ml) to 10 µg D-sorbitol or xylitol/assay (0.1 g D-sorbitol or xylitol/l sample solution; \(v = 0.100\) ml).
Precision: - In a double determination of D-galactose using one sample solution, a difference of 0.005 to 0.010 absorbance units may occur. The relative standard deviation is approx. 1% to 2% in the measuring range.

Samples Per Kit: - 3 x 12 tests.

Product ID #: - E0670057.