2E-014

Löffler’s methylene blue, alkaline

In-vitro diagnostic agent

Description

The product 2E-014 is a ready-to-use solution for professional users for application in histology. It is an aqueous dye solution mixed with potassium hydroxide. The product comes in 5 different pack sizes: 2E-014.00100 (100ml bottle), 2E-014.00250 (250ml bottle), 2E- 014.00500 (500ml bottle), 2E-014.01000 (1l. bottle) and 2E-014.10000 (10l. canister)

Main components

Methylene blue (CI 52015)	2.5 g/l
Potassium hydroxide (CAS 1310-58-3)	0.15 g/l
Ethanol (CAS 64-17-5) 30%	

Purpose

"Löffler’s methylene blue solution" is used for cell diagnostics for the examination of microbacteria, fungi and blood parasites and as a vital dye for the selective staining of cell nuclei (e.g. counting of eukaryotic cells). It is a ready-to-use dye solution for professional users, often used in combination with the carbol-fuchsin solution in Ziehl-Neelsen staining. It can be used for staining microscopic specimens in microbiology to distinguish acid-resistant bacteria from other non-acid-resistant bacteria by staining.

Sample material and sample preparation

Sampling may only be carried out by qualified personnel. All samples must be processed with state-of-the-art technology. All samples must be clearly labelled.

Sample material: Smear specimens after air drying, heat fixation and pre-treatment with Sputoflol®, such as sputum, smears from fine needle aspiration biopsies, blood smears, rinsing fluids, imprints, impressions, pus, exudates, effusions after air drying, heat fixation and pre-treatment with Sputoflol®.

Test principle

Staining using Löffler’s methylene blue solution is suitable for direct staining of cell material, bacteria and fibres. The staining is selective so that the grey matter in the peripheral nervous system can be stained.

The cell wall of acid-resistant bacteria absorbs dyes very slowly due to the high proportion of wax and lipids, thus preventing the bacteria from staining. The principle of Ziehl-Neelsen staining is initially based on staining with carbol fuchsin under heat. This allows the dye to penetrate despite the lipid envelope. The specimens are then treated with hydrochloric acid or a mixture of ethanol and hydrochloric acid at normal temperature, and only the acid-resistant bacteria retain the dye and stay red. All non-acid-resistant microorganisms are counterstained with Löffler’s methylene blue solution.

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Staining

Prior to staining, the specimens should be treated with Sputofluol® solution to loosen them from viscous sputum and cellular material. The Löffler's methylene blue dye solution enables direct staining of the samples and makes bacteria and cell material appear blue.

Within the staining with carbolfuchsin solution according to Ziehl-Neelson, counterstaining is done with Löffler's methylene blue for 30 seconds followed by another rinse under running tap water. The samples are transferred to xylene via an ascending ethanol series. The samples can be covered with a synthetic covering medium for subsequent examination under a microscope.
To ensure the differentiability of the target structures, suitable control specimens should be kept along with the staining.

The usual staining protocols known from literature must be used.
Staining may only be carried out by qualified personnel.

Result

Living cell material (cell walls, nuclei), bacteria, fibres	blue

Precautionary measures

When removing the product, care must be taken to avoid contamination of the storage vessel. Once the solution has been removed, it must not be returned to the canister. If turbidity or solids appear, discard the product. The product is intended for single use and must not be reused.

Storage and shelf life

Store the unopened containers in a dry place at 15 to 25 °C, avoiding direct sunlight.
The shelf life is 2 years. See also the best-before date (BBD) on the label. Once the containers have been opened, the shelf life corresponds to the best-before date, as long as the storage conditions are observed and the solution is handled properly.

Safety notice

If any serious incidents occur in connection with the product, please report them to the manufacturer and the national authority.

Literature

Romeis, Mikroskopische Technik, Editors: Maria Mulisch, Ulrich Welsch, 2010, Springer Spektrum, 18th edition

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