



## 2C-176

Methylene blue solution (1%), alcoholic  
In-vitro diagnostic agent

### Description

The product 2C-176 is a ready-to-use solution for professional users for application in histology. The product comes in 4 different pack sizes: 2C-176.00100 (100ml bottle), 2C-176.00250 (250ml bottle), 2C-176.01000 (1l bottle) and 2C-176.10000 (10l canister).

### Main components

Methylene blue (C.I. 52015)      10g/l

### Purpose

"Methylene blue solution (1%, alcoholic)" is used for cell diagnostics for the examination of microbacteria. It is a ready-to-use dye solution for professional users. 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, rinsing fluids, imprints, effusions, pus, exudates, liquid and solid cultures as well as sections of human tissue (3–4 µm thickness) after fixation, for instance by means of buffered formol and fixation mixtures with ethanol and formalin and subsequent embedding in paraffin.

### Test principle

The cell wall of acid-resistant bacteria absorbs dyes very slowly due to the high proportion of wax and lipids and prevents the bacteria from staining. The principle of Ziehl-Neelsen staining is based on staining with carbolfuchsin under heat. This allows the dye to penetrate despite the lipid envelope. The specimens are then decolourised with hydrochloric acid or a mixture of ethanol and hydrochloric acid at normal temperature; only the acid-resistant bacteria retain the dye and stay red. Counterstaining with methylene blue solution stains all non-acid-fast microorganisms.

### Staining

Prior to staining, the specimens should be pre-treated with Sputofluol® solution to loosen them from tough sputum and cellular material. Staining with carbol fuchsin solution according to Ziehl-Neelsen is carried out on dry, dust-free and heat-fixed specimens. After the solution is dripped on, the specimen is heated three times until steam is formed for a total of 5 minutes. After staining with carbol-fuchsin according to Ziehl-Neelsen, the samples are rinsed under running tap water until no more colour clouds come off. The samples are rinsed in hydrochloric acid-ethanol solution for 15-30 seconds until no more colour clouds come off and then washed under running tap water. Counterstain with methylene blue solution for 30 seconds and rinse again 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

Acid-fast bacteria	red
Background	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 5 to 30 °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