Instructions for use

Ziehl-Neelsen Staining Kit

8276

Detection of acid-resistant bacteria

Acid-resistant bacteria are, for example, mycobacteria such as *M. tuberculosis* or *M. leprae*. They have special lipids (mycolacids) in their cell walls which give them hydrophobic properties. They therefore cannot be stained with the usual hydrophilic dyes.

To pass this barrier, the staining solution must be heated until it forms steam (do not boil). The wax coating "melts" and the dye can penetrate. A very stable dye complex (mycolate-fuchsin complex) forms in the cell wall. After cooling down, the bacteria can hardly be decoloured again even by intensive differentiation agents such as HCl alcohol. While the dye is washed out of the remaining tissue components, the acid-resistant bacteria remain stained and can thus be detected.

With *carbolic fuchsine solution* acid-resistant bacteria are stained red under the influence of heat.

Löffler's methylene blue solution is used for counterstaining at room temperature. It stains all other cell components blue.

Kit contains

- Carbolic fuchsine solution (Art. No. A130.2) 500 ml
  Danger H314-H341
- Löffler‘s methylene blue solution (Art. No. AE64.1) 500 ml

The staining solutions should be filtrated before use! Solutions may be bought separately.

Additional chemicals required:
HCl-ethanol solution 3 % (Art. No. 6477) – working solution 0.5 %

Instruction for paraffin sections*:

1. Dewax and rehydrate the fixed section. *Do not use a fixing medium that removes lipids out of the tissue (e.g. Carnoy).*	5. Decant the staining solution and rinse with tap water for a short time.
2. Cover the section with carbolic fuchsine solution.	6. Differentiate with HCl-ethanol solution until no more colour runs off. 1-3 min
3. Heat the slide on the Bunsen burner until vapour releases (do not boil). Repeat the process 2 times. Cool down the specimen in between. *Caution! Toxic vapours!*	7. Rinse with tap water in order to stop the differentiation. *Examine by microscope*: *Only mycobacteria may be stained red!*
4. Incubate in carbolic fuchsine solution (60 °C). 1 – 24 h Let the specimen cool down in the staining solution.	8. Rinse the acid well with flowing tap water.
	9. Counterstain with Löffler‘s methylene blue solution. ca. 1 min Rinse with distilled water.

*Acc. to Romeis, Mikroskopische Technik, 18. Auflage, Spektrum Akademischer Verlag (2010)

Result:

- Acid-resistant bacteria: red
- Other bacteria and microorganisms: blue
- Tissue: light blue

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Instruction for cytological specimens*:

1. Fix the specimen by heat on the slide.	5. Differentiate with HCl-ethanol solution until no more colour runs off. 1-3 min
2. Cover with carbolic fuchsine solution.	6. Rinse the acid well with tap water.
3. Heat the slide on the Bunsen burner until vapour releases (do not boil). Repeat the process 2 times. Cool down the specimen in between. *Caution! Toxic vapours!*	7. Counterstain with Löffler's methylene blue solution. ca. 1 min
4. Decant the staining solution and rinse with tap water for a short time.	8. Rinse well with distilled water.
	9. Let specimen air-dry.

*Acc. to Romeis, Mikroskopische Technik, 18. Auflage, Spektrum Akademischer Verlag (2010)

Result:

- Acid-resistant bacteria: red
- Background: light blue

Please Note:
With this method it is only possible to prove the existence of acid-resistant bacteria.
More reliable statements about the bacteria – e.g. kind and state - are not possible.

Please Note:
The colour intensity depends on the pre-treatment and the composition of the samples to be stained.
It may initially be necessary to adapt the method to the respective conditions.

Ziehl-Neelsen staining kit	**1 Kit**	**Glass**	**8276.1**

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