

Fixing

In most cases, staining with appropriate dyes is necessary to make microorganisms and their details visible under the microscope.

Bacteria staining, apart from supravital staining (e.g. fluorescence stains), is carried out on fixed cells. Particularly heat sensitive bacteria are merely air dried.

1. Heat fixing

Reagent

<i>Merck Cat. No.</i>	<i>Product</i>	<i>Pack size</i>
1.06408.0100	Sodium chloride tablets	100 tablets

Solution

Physiological sodium chloride solution: Dissolve 1 sodium chloride tablet in 1 litre of demineralized water.

[Experimental Procedure and Evaluation](#)

Experimental Procedure and Evaluation

Using a loop, place the specimen on a fat free slide and streak either directly (e.g. viscous exsudate, puss or liquid cultures) or after dilution with physiological sodium chloride solution (e.g. centrifuge sediment, pure cultures from solid culture media). After complete air drying, or for the sake of speed after careful heat drying, fix by slowly moving the slide in a circle of about 2.5 cm three times through the dark flame of a Bunsen burner (the specimen side of the slide should be at the top).

2. Chemical fixing

In contrast to heat fixing, chemical fixing permits a better contrasted visualisation of bacteria details e.g. the cilia or the relationship between bacteria and body cells.

Solutions

1.	Ethanol ether: Ethanol abs. 50 ml; diethyl ether 50 ml
2.	Sublimate alcohol: Mercury(II)chloride 3 g; demin. water to 60 ml; ethanol abs. 30 ml.

3.	Osmic acid solution: Osmic acid solution 2 % 5 ml; conc. acetic acid 5 drops. Store in a bottle with a wide neck.
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Experimental Procedure and Evaluation

Cover specimens with the following fluids or lay specimens in the baths.

- a) methanol 2-3 minutes
- b) or ethanol ether (1) 10-15 minutes
- c) or sublimate alcohol (2) 3-5 minutes
- d) It is also possible to hold the slide above hot steaming water and then place the wet slide on the opening of the wide neck bottle (3) to impregnate it with osmic acid fumes.

Merck Cat.No.	Product	Pack Size
1.00062.1000	Acetic acid min. 96 %	1 l
1.00921.1000	Diethyl ether	1 l
1.00983.1000	Ethanol absolute	1 l
1.04419.0050	Mercury(II) chloride	50 g
1.06009.1000	Methanol (decolourizer)	1 l
1.09266.0005	Osmic acid solution 2 % 5 ml	

Reagents

