

# ZIEHL NEELSEN KIT

Kit for colouring acid-alcohol resistant bacteria

## DESCRIPTION

ZIEHL NEELSEN KIT is a kit for colouring acid-alcohol resistant bacteria present in pathological samples or in culture media. This colouring is particularly useful is performing a microscope examination for the presence of mycobacteria.

## CONTENT OF THE PACKAGES

The reagents are contained in plastic bottles, closed by thermal induction and provided with a dropper lid.

Each pack contains:

- 1 bottle containing 250 ml of Fuchsin Solution
- 1 bottle containing 250 ml of Alcohol–Acid Solution
- 1 bottle containing 250 ml of Methylene Blue Solution
- 1 Instruction sheet

## PRINCIPLE OF THE METHOD

ZIEHL NEELSEN colouring is based on the fact that phenol allows basic Fuchsin to penetrate the interior of the bacterial cell. After they have been stained with basic Fuchsin, some bacteria remain red if treated with the Acid-Alcohol Solution (acid-alcohol resistant bacteria). Others lose the red colour and take up methylene blue, becoming blue in colour (bacteria sensitive to decolouring with acid-alcohol).

## COMPOSITION

The formulation of the contents of the ZIEHL NEELSEN KIT is that envisaged for colouring mycobacteria. The three solutions included in the kit are all aqueous solutions, except, naturally, for the Acid-Alcohol Solution.

Table no.1

Ziehl Neelsen Kit Content			
1	Fuchsin solution	Basic Fuchsin Ethyl alcohol 95% Phenol	0.3% 10% 5%
2	Acid-alcohol solution	Ethyl alcohol 95% HCl Concentrated	97% 3%
3	Methylene Blue Solution	Methylene Blue	0.3%

## COLLECTION OF SAMPLES

The samples to be subjected to acid-alcohol resistant colouring are principally clinical material and microbial cultures.

## TEST PROCEDURE

### Preparation and fixing

On clean slides make a smear of the pathological material or of the culture. Leave to dry in the air and fix by passing rapidly over the flame, or by using other fixing methods. Do not overheat the sample when fixing.

### Colouring

In all phases of the colouring, the preparation must be rinsed with water free of acid-alcohol resistant species.

1. Cover the slide with the Fuchsin Solution. Heat the slide slowly until steam forms. Continue heating the slide for 3-5 minutes. Leave to cool and then rinse with water.
2. Decolour with the Acid-Alcohol Solution for as long as the preparation releases colour (about 1-2 minutes). Rinse with water.
3. Cover the slide with Methylene Blue Solution. Leave in contact for 30-60 seconds. Then rinse with water and leave to dry.
4. Examine the preparation under the microscope with the objective for immersion.

## INTERPRETATION OF THE RESULTS

The mycobacteria and acid-alcohol resistant bacteria appear coloured red, whereas the background material and other non acid-alcohol resistant material are coloured blue.

## QUALITY CONTROL

Each lot of ZIEHL NEELSEN KIT is subjected to quality control using a culture of *Mycobacterium smegmatis* ATCC 14468 for the test for acid-alcohol resistant bacteria (red colouring) and a culture of *Staphylococcus aureus* ATCC 25923 for the test for bacteria sensitive to decolouring with acid-alcohol (blue colouring).

## LIMITS

1. ZIEHL NEELSEN colouring provides presumptive evidence of the presence of acid-alcohol resistant micro-organisms in the sample, evidence that must be confirmed using other methods. A negative result for the colouring does not necessarily prove that the sample is negative for the presence of acid-alcohol resistant micro-organisms.
2. Fast growing mycobacteria may retain the colouring to different degrees.
3. When decolouring with the Acid-Alcohol Solution, take care to avoid insufficient decolouration.

## PRECAUTIONS

The ZIEHL NEELSEN KIT package contains substances classified as hazardous by current legislation. It is recommended that the Safety Data Sheets be consulted on their use. ZIEHL NEELSEN KIT is a kit for bacteria colouring, only for diagnostic use *in vitro*. It is intended for use in a professional environment and must be used in a laboratory by adequately trained personnel using approved asepsis and safety methods for dealing with pathogenic agents.

## STORAGE

Store the ZIEHL NEELSEN KIT at 10-25°C in its original packaging. Keep away from sources of heat and avoid excessive changes of temperature.

In such conditions the product ZIEHL NEELSEN KIT will remain valid until the expiry date indicated on the label. Do not use beyond that date.

Eliminate without using if there are signs of deterioration (change in the colour of the solutions or presence of substantial precipitates).

## DISPOSAL OF USED MATERIAL

After use, the slides coloured with the ZIEHL NEELSEN KIT and any material that has come into contact with the sample must be decontaminated and disposed of in accordance with the techniques used in the laboratory for decontamination and disposal of potentially infected material.

## PRESENTATION

Product	REF	
ZIEHL NEELSEN KIT	80276	3 x 250 ml

## TABLE OF SYMBOLS

<b>IVD</b> In Vitro Diagnostic Medical Device	 Do not reuse	 Manufacturer	 Contains sufficient for <n> tests	 Temperature limitation
<b>REF</b> Catalogue number	 Fragile, handle with care	 Use by	 Caution, consult accompanying documents	<b>LOT</b> Batch code