

## TECHNICAL LEAFLET

**Differentiation of acid fast micro-organisms in smears & sections.****Principle of method**

This kit contains all reagents required to carry out the technique of Kinyoun et al, for the identification of acid fast organisms in smears or tissue sections.

Each kit contains the following reagents:

Carbol fuchsin -KY	250ml
Counterstain	250ml
TB-Differentiator solution	2 x 250ml

**Storage & stability**

Keep the reagents in the kit packaging provided and ensure that the nozzles are closed to prevent evaporation & oxidation from the air. Store the kit at room temperature in a darkened cupboard. The kit reagents will remain stable in these conditions. This kit is for "In-vitro" diagnostic use only.

**Preparation of reagents and usage information**

To prepare the reagent bottles for use, using scissors or similar device cut off the top 3mm of the nozzle or pierce the top of the nozzle with a nail or similar to make a small hole. Ensure that the nozzle cap will seal the nozzle. If an excessive amount is cut off you may not be able to re-seal the nozzle, if this occurs contact us for replacement nozzles.

In use carefully apply the nozzle to the slide and squeeze out sufficient reagent to cover the sample. Excessive pressure is not required to release the reagent, ensure that the opening in the nozzle is sufficient to allow the reagent to flow without excessive pressure being applied. The reagents are now ready to use.

Use a known positive control with the test slide to confirm that the reagent system is working.

Information obtained from stained slides in concentration procedures should always be backed up by culturing techniques.

**Method**

Prepare a 3-4 sq cm thin smear with as little mechanical manipulation as possible. Excessive movement may cause damage to bacterial cells and loss of acid fastness. Heat fix the smear by passing the slide, smear side up, through a Bunsen flame 5-6 times.

1 - Flood the smear with KY Carbol fuchsin for 5-15 minutes at room temperature. For thick smears and sections stain for up to 30 minutes at room temperature.

2 - After staining rinse in tap water 5-10 seconds.

3 - Decolourise by running the TB-Differentiator solution over the smear. The decolourisation time will vary with the thickness of the smear, generally 5-30 seconds for thin smears, up to 2 minutes for thick smears and upto 5 minutes for sections until the tissue is pink- colourless. After decolourisation, rinse briefly in tap water.

4 - Flood slide with counterstain 10-20 seconds. Rinse well in tap water and examine.

The method given in this leaflet is designed as a general guide only and should be modified to suit the samples being processed to obtain optimal staining & decolourisation times.

**Results**

Acid fast organisms	bright red / pink
Non acid-fast organisms & background material	blue / green

GCC Diagnostics guarantees that the highest quality reagents are supplied with this product to give reliable results within the lifetime of this kit stored as directed. The user should however, determine the suitability of this product for their intended use.

**Unsatisfactory performance**

As part of our duty to monitor product performance and our policy of continual improvement

Please report to us any unsatisfactory performance you may experience with this product. In the event of any reagent degrading before expiry of shelf life please contact us for a replacement

**MSDS Section**

KY-Carbol fuchsin contains organic dyestuffs <1%, Alcohols <15% Glycols <15% Phenol <5% , balance water.

TB Differentiator contains mineral acid <3%, Alcohols <50% , balance water.

Methylene blue counterstain contains organic dyestuffs <2%, glycols <10%, balance water.

Malachite green counterstain contains organic dyestuffs <2%, glycols <10%, balance water.

When using this product observe the usual rules for the safe handling and use of laboratory reagents, wear suitable body, face and eyes protection. Ensure eye bath station is nearby. There is a potential danger of liquid reagent squirting out of the nozzle of the bottle by accident or in mis-use, be aware of this potential danger. Keep top on reagent bottles at all times when not in use.

KY-Carbol fuchsin - contains phenol which is TOXIC and can be absorbed through the skin and by inhalation and ingestion. H301+311+332 Toxic if swallowed, by skin contact and inhalation. H319 Causes serious eye irritation. P260 Do not breathe vapour. P262 Do not get in eyes, skin, clothing. P285 In case of inadequate ventilation, wear respiratory protection. IF SWALLOWED - rinse mouth with water, seek urgent medical attention. IF INHALED - move to clear air zone. IF ON SKIN or in EYES - rinse with soap and water and apply eye bath. P314 Get medical attention if you feel unwell.

TB Differentiator - H226 Flammable irritant liquid and vapour  
H302+H312+H320 Harmful if swallowed, by skin contact and inhalation. P233 Keep container tightly closed. P210 Keep away from sources of ignition . P262 Do not get in eyes, skin, clothing.  
IF SWALLOWED - rinse mouth with water, seek urgent medical attention. IF INHALED - move to clear air zone. IF ON SKIN or in EYES - rinse with soap and water and apply eye bath. Seek medical attention if you feel unwell.

Methylene blue/ Malachite green counterstain - H304+313 Harmful if swallowed, enters airways by skin contact .  
Keep container tightly closed. P262 Do not get in eyes, skin, clothing.  
IF SWALLOWED - rinse mouth with water, seek urgent medical attention. IF INHALED - move to clear air zone. IF ON SKIN or in EYES - rinse with soap and water and apply eye bath. P314 Get medical attention if you feel unwell.

#### **Waste disposal of reagents**

All reagents in this kit may be disposed of down the public sewer system by dilution with water, however check with local sewerage authority about the disposal of small amounts of phenolic residues.

The cleaned empty packaging should be recycled as polyethylene , cardboard, paper.

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