

### INTENDED USE

For use in staining smears prepared from clinical specimens suspected of containing Mycobacteria.

### SUMMARY AND EXPLANATION

The Kinyoun Carbol Fuchsin staining technique is a variation of the acid-fast method developed by Robert Koch in 1882. Mycobacteria possess unique acid-fast characteristics that make the acid-fast staining techniques invaluable in detecting Mycobacteria species.

### PRINCIPLE OF THE TEST

The lipid content of the cell wall of acid-fast bacilli makes staining of the organisms difficult. If an organism is to be termed 'acid-fast' it must resist decolourisation by acid alcohol. A counterstain is then used to emphasise the stained organism. The high concentration of phenol in the Kinyoun Carbol Fuchsin facilitates penetration of the stain, and allows retention in the cell wall even after exposure to decolourisers.

### MATERIALS PROVIDED

#### Ready to use Stains and Differentiators:

|   |             |                          |         |
|---|-------------|--------------------------|---------|
| - | PL.7021/25  | Kinyoun Carbol Fuchsin   | 250 ml  |
| - | PL.7021     | Kinyoun Carbol Fuchsin   | 500 ml  |
| - | PL.7022     | Kinyoun Carbol Fuchsin   | 1000 ml |
| - | PL.7024/100 | Diff for ZN & Kinyoun CF | 100 ml  |
| - | PL.7024/25  | Diff for ZN & Kinyoun CF | 250 ml  |
| - | PL.7024     | Diff for ZN & Kinyoun CF | 500 ml  |
| - | PL.7025     | Diff for ZN & Kinyoun CF | 1000 ml |
| - | PL.7026     | Diff for ZN & Kinyoun CF | 2000 ml |
| - | PL.7027/100 | Methylene Blue           | 100 ml  |
| - | PL.7027/25  | Methylene Blue           | 250 ml  |
| - | PL.7027     | Methylene Blue           | 500 ml  |
| - | PL.7028     | Methylene Blue           | 1000 ml |
| - | PL.7029     | Methylene Blue           | 2000 ml |
| - | PL.7030/100 | Malachite Green          | 100 ml  |
| - | PL.7030/25  | Malachite Green          | 250 ml  |
| - | PL.7030     | Malachite Green          | 500 ml  |
| - | PL.7031     | Malachite Green          | 1000 ml |
| - | PL.7032     | Malachite Green          | 2000 ml |

#### Per 100ml solution:

- Kinyoun Carbol Fuchsin contains 2.95g Basic Fuchsin powder.
- Diff for ZN and Kinyoun CF contains 3ml of Hydrochloric Acid.
- Ready to use Methylene Blue contains 0.4g of Methylene Blue powder.
- Ready to use Malachite Green contains 0.4g of Malachite Green powder.

#### Concentrated Stains (dilute 1 part in 10 with deionised or reverse osmosed water before use):

|   |             |                 |       |
|---|-------------|-----------------|-------|
| - | PL.8006     | Methylene Blue  | 100ml |
| - | PL.8006/4.0 | Methylene Blue  | 400ml |
| - | PL.8006/5.0 | Methylene Blue  | 500ml |
| - | PL.8007     | Malachite Green | 100ml |
| - | PL.8007/4.0 | Malachite Green | 400ml |
| - | PL.8007/5.0 | Malachite Green | 500ml |

#### Per 100ml solution:

- Concentrated Methylene Blue contains 4g of Methylene Blue powder.
- Concentrated Malachite Green contains 4g of Malachite Green powder.

### MATERIALS REQUIRED BUT NOT PROVIDED

- Glass slides
- Inoculating loops
- Microscope
- Immersion Oil PL.396
- Pro-Slide™ Acid-Fast Stain Control PL.4960

### STABILITY AND STORAGE

The stains and differentiators should be stored at 15-25°C in their original containers. Product stored under these conditions will be stable until the expiry date shown on the product label.

### PRECAUTIONS

- For *In Vitro* Diagnostic Use only.
- For professional use only.
- Directions should be read and followed carefully.
- Do not use beyond the stated expiration dates.
- Microbial contamination may decrease the accuracy of the staining.
- Safety precautions should be taken in handling, processing and discarding all clinical specimens.
- Samples should be processed in the correct containment level conditions.
- Dispose of all material in accordance with local regulations.

### TEST PROCEDURE

1. Prepare a smear on a clean glass slide and allow to air dry.
2. Heat fix and allow to cool.
3. Flood the slide with Kinyoun Carbol Fuchsin, stand for 10 minutes.
4. Rinse with water.
5. Flood the slide with Differentiator for ZN & Kinyoun Carbol Fuchsin for 10 minutes, applying a change of differentiator at 5 minutes.
6. Rinse with water.
7. Flood the slide with counterstain (Methylene Blue or Malachite Green), stand for 1 minute.
8. Rinse well with water; gently blot dry or dry using gentle heat.
9. Examine using a microscope.

### QUALITY CONTROL PROCEDURE

Internal quality control of the stains and differentiators must be performed regularly on known reference material.

#### Recommended quality control:

Positive control – *Mycobacterium scrofulaceum* NCTC® 10803/ATCC® 19981\*  
 Negative control – *Escherichia coli* NCTC® 12241/ATCC® 25922\* (PLD02)  
 Pro-Slide™ Acid-Fast Stain Control PL.4960

### INTERPRETATION OF RESULTS

Acid-fast bacilli are stained a pink-red colour. Other organisms are stained blue or green depending on the counterstain used.


### LIMITATIONS OF THE PROCEDURE


- Only experienced personnel should carry out the interpretation of stained slides.
- Read prepared slides as soon as possible after staining. Failure to do so may affect the results.
- False staining results can be seen due to cellular debris being stained by the technique.


- Positive staining reactions provide presumptive evidence of the presence of *Mycobacteria* in the specimen only. Negative staining results do not necessarily indicate the specimen will be negative on culture. Culture methods should also be employed for positive identification of Mycobacteria.
- Organisms other than *Mycobacteria* may display varying degrees of acid-fastness e.g. *Rhodococcus* spp., *Cryptosporidium* spp., and *Isopora* spp.


### REFERENCES


- Cruickshank, R., Duguid, J. P., Marmion, B. P. and Swain, R.H.A. The Practice of Medical Microbiology. 12th Edition. V2
- Kinyoun, J.J. 1915. A note on Uhlenhuth's method for sputum examination for tubercle bacilli. *American Journal of Clinical Pathology*. 46:472-4.
- Lennette. 1974. Manual of Clinical Microbiology. *American Society for Microbiology*, Washington, D.C.
- Neelson, F. 1883. Ein Casuistischer Beitrag zur Lehre von der Tuberkulose. *Centralbl. Med. Wiss.* 21:497-501.
- Public Health England. May 2019. UK Standards for Microbiology Investigations: Staining Procedures. *Bacteriology – Test Procedures*. TP 39, Issue no.3.
- Ziehl, F. 1882. Zur Färbung des Tuberkelbacillus. *Dtsch. Med. Wochenschr.* 8:451.


 = Use by


 = Lot number


 = Catalogue number


 = Manufacturer

 = Authorized Representative in the European Community

 = Contains sufficient for <n> tests

 = In vitro diagnostic medical device

 = Temperature limitation

 = Consult instructions for use

EC
REP






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## HAZARDS IDENTIFICATION

Please refer to Safety Data sheets for full text for all hazard and precautionary statements.

|   |  |  |
|---|--|--|
|  <p>DANGER</p>   | PL.7021/25<br>PL.7021<br>PL.7022   | H226, H302+H332, H314, H341,<br>H351, H373, H412<br><br>P210, P270, P273, P280,<br>P301+P330+P331,<br>P303+P361+353, P310,<br>P305+P351+P338, P501   |
|  <p>DANGER</p>   | PL.8007<br>PL.8007/4.0<br>PL.8007/5.0  | H226, H318, H361, H411<br><br>P210, P273, P280,<br>P303+P361+P353,<br>P305+P351+P338, P310, P501   |
|  <p>DANGER</p>   | PL.7024/100<br>PL.7024/25<br>PL.7024<br>PL.7025<br>PL.7026<br><br>PL.7027/100<br>PL.7027/25<br>PL.7027<br>PL.7028<br>PL.7029 | H225, H332, H319, H371<br><br>P210, P270, P280,<br>P303+P361+P353, P304+P340,<br>P305+P351+P338, P312, P501<br><br>H226, H332, H370<br><br>P210, P270, P280,<br>P303+P361+P353, P304+P340,<br>P312, P501 |
|  <p>WARNING</p> | PL.7030/100<br>PL.7030/25<br>PL.7030<br>PL.7031<br>PL.7032   | H226, H319, H412<br><br>P210, P280, P305+P351+P338,<br>P337+P313, P370+P378, P501  |
|  <p>DANGER</p> | PL.8006<br>PL.8006/4.0<br>PL.8006/5.0  | H226, H302, H311+H331, H370<br><br>P210, P270, P280, P301+P310,<br>P330, P304+P340, P311, P501   |