Acid-Fast Stains for Mycobacteria (Kinyoun Carbol Fuchsin)

(for *In Vitro* Diagnostic use only)

PRODUCT CODE: SEE MATERIALS PROVIDED

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INTENDED USE

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

SUMMARY AND EXPLANATION

The Kinyoun carbol fuchsin stain 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.

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

Acid-fast stains for Mycobacteria should be stored at 15-25ºC 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.

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 CF 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

Internal quality control of the Kinyoun carbol fuchsin stains 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

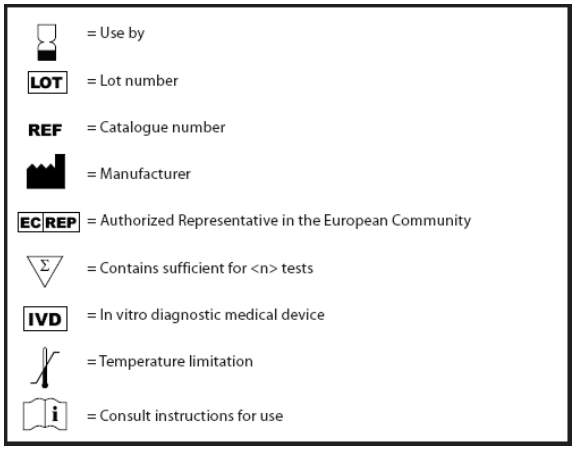
Acid-fast bacilli are stained red. Other organisms are stained blue or green dependent on the counterstain used.

LIMITATIONS

- 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 *M. tuberculosis* in the specimen only.
- Negative staining results do not necessarily indicate the specimen will be negative on culture.
- 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. Manual of Clinical Microbiology. *American Society for Microbiology*, Washington, D.C. 1974.
- Neelson, F. 1883. Ein Casuistischer Beitrag zur Lehre von der Tuberkulose. *Centraldl. Med. Wiss.* 21:497-501.
- Ziehl, F. 1882. Zur Farbung des Tuberkelbacillus. *Dtsch. Med. Wochenschr.* 8:451.



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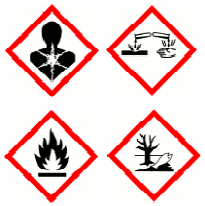
HAZARDS IDENTIFICATION

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

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