ACID-FAST STAINS FOR MYCOBACTERIA (ZN CARBOL FUCHSIN)

(for *In Vitro* Diagnostic use only)

PRODUCT CODE: See materials provided

CE

INTENDED USE

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

SUMMARY AND EXPLANATION

The ZN carbol fuchsin stain is a variation of the acid-fast method developed by Robert Koch in 1882. Mycobacteria possess unique acid fast characteritics that make the acid-fast staining techniques invaluable in detecting Mycobacteria species.

PRINCIPLE

The lipid content of the cell wall of acid-fast bacilli makes staining of the organisms difficult. In the modified Ziehl-Neelson staining method, the phenol component of the kinyoun carbol fuchsin stain allows the stain to penetrate even after exposure to decolourisers. 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.7018/100	ZN Carbol Fuchsin	100 ml
PL.7018/25	ZN Carbol Fuchsin	250 ml
PL.7018	ZN Carbol Fuchsin	500 ml
PL.7019	ZN Carbol Fuchsin	1000 ml
PL.7020	ZN Carbol Fuchsin	2000 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

Staining Kits (ready to use):

PL.8060/25 TB Staining Kit (Methylene Blue)
1 x PL.7018/25, 2 x PL.7024/25, 1 x PL.7027/25

PL.8061/25 TB Staining Kit (Malachite Green)
1 x PL.7018/25, 2 x PL.7024/25, 1 x PL.7030/25

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

PL.8005	ZN Carbol Fuchisn	100 ml
PL.8005/4.0	ZN Carbol Fuchisn	400 ml
PL.8005/5.0	ZN Carbol Fuchisn	500 ml
PL.8006	Methylene Blue	100 ml
PL.8006/4.0	Methylene Blue	400 ml
PL.8006/5.0	Methylene Blue	500 ml
PL.8007	Malachite Green	100 ml
PL.8007/4.0	Malachite Green	400 ml
PL.8007/5.0	Malachite Green	500 ml

MATERIALS REQUIRED BUT NOT PROVIDED

- Glass slides
- Innoculating loops
- Microscope
- Immersion oil PL.396
- AFB QC slides PL.4960

STABILITY AND STORAGE

Acid-fast stains for Mycobateria 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 ZN carbol fuchsin and heat gently (do not boil). Allow to stand for 10 minutes applying heat again after 5 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 gente heat.
9. Examine using a microscope.

QUALITY CONTROL PROCEDURE

Internal quality control of the ZN carbol fuchsin stains must be performed regularly on known reference material.

Recommended quality control:

Positive control – A proven positive

Negative control - A proven negative

CE marked QC slides – PL.4960

INTERPRETATION OF RESULTS

Acid-fast bacilli are stained red. Other organisms are stained blue or green dependent 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 celular debris being stained by the trechnique.
- 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. Culture methods should also be employed for positive identification of *M. tuberculosis*.
- Organisms other than mycobacteria may dispaly varying degrees of acid fastness e.g. *Rhodococcus* spp., *Cryptosporidium* spp., and *Isopora* spp.

REFERENCES

- Ziehl, F. 1882. Zur Farbung des Tuberkelbacillus. Dtsch. Med. Wochenschr. 8:451.
- Neelson, F. 1883. Ein Casuistischer Beitrag zur Lehre von der Tuberkulose. Centraldl. Med. Wiss. 21:497-501.
- Kinyoun, J. J. 1915. A note on Uhlenhuth's method for sputum examination for tubercle bacilli. Am. J. Clin. Pathol. 46:472-4.
- Manual of Clinical Microbiology. Lennette.
- The Practice of Medical Microbiology. 12th Edition. V2. R. Cruickshank, J. P. Duguid, B. P. Marmion, R.H.A. Swain.

Revision 2021 03

Canada 800 268 2341 Fax 905 731 0206
20 Mural Street, Unit 4, Richmond Hill, ON, L4B 1K3

U.S.A. (512) 832 9145 Fax (512) 832 6424
21 Cypress Blvd, Suite 1155, Round Rock, Texas, 78665-1046

UK 0151 353 1613 Fax 0151 353 1614
3 Bassendale Road, Bromborough, Wirral, Merseyside, CH62 3QL

ACID-FAST STAINS FOR MYCOBACTERIA (ZN CARBOL FUCHSIN)

(for *In Vitro* Diagnostic use only)

PRODUCT CODE: See materials provided

CE

= Use by

LOT = Lot number

REF = Catalogue number

= Manufacturer

EC REP = Authorized Representative in the European Community

= Contains sufficient for <n> tests

IVD = In vitro diagnostic medical device

= Temperature limitation

= Consult instructions for use

EC REP

Advena Ltd. Tower Business Centre, 2nd Floor,
Tower Street, Swatar, BKR 4013, Malta.
T: +44(0) 1926 800 153

HAZARDS IDENTIFICATION

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

 DANGER	PL.7018/100 PL.7018/25 PL.7018 PL.7019 PL.7020	H226, H302, H314, H341, H351, H412 P210, P273, P280, P301+330+P331, P303+P361+P353, P305+P351+P338, P310, P501
 DANGER	PL.8005 PL.8005/4.0 PL.8005/5.0	H226, H301+H331, H312, H314, H341, H351, H373, H411 P210, P273, P280, P301+330+P331, P310, P302+P352, P304+P340, P305+P351+P338, P501
 DANGER	PL.7024/100 PL.7024/25 PL.7024 PL.7025 PL.7026	H255, 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
 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
 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.8007 PL.8007/4.0 PL.8007/5.0	H226, H318, H361, H411 P210, P273, P280, P303+P361+P353, P305+P351+P338, P310, P501

Canada 800 268 2341 Fax 905 731 0206
20 Mural Street, Unit 4, Richmond Hill, ON, L4B 1K3

U.S.A. (512) 832 9145 Fax (512) 832 6424
21 Cypress Blvd, Suite 1155, Round Rock, Texas, 78665-1046

UK 0151 353 1613 Fax 0151 353 1614
3 Bassendale Road, Bromborough, Wirral, Merseyside, CH62 3QL