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 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 allow penetration of the stain, and resist decolourisation by acid alcohol. A counterstain is then used to emphasise the stained organism. The high concentration of phenol in the ZN 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.7018/100	ZN Carbol Fuchsin	100ml
-	PL.7018/25	ZN Carbol Fuchsin	250ml
-	PL.7018	ZN Carbol Fuchsin	500ml
-	PL.7019	ZN Carbol Fuchsin	1000ml
-	PL.7020	ZN Carbol Fuchsin	2000ml
-	PL.7024/100	Diff for ZN & Kinyoun CF	100ml
-	PL.7024/25	Diff for ZN & Kinyoun CF	250ml
-	PL.7024	Diff for ZN & Kinyoun CF	500ml
-	PL.7025	Diff for ZN & Kinyoun CF	1000ml
-	PL.7026	Diff for ZN & Kinyoun CF	2000ml
-	PL.7027/100	Methylene Blue	100ml
-	PL.7027/25	Methylene Blue	250ml
-	PL.7027	Methylene Blue	500ml
-	PL.7028	Methylene Blue	1000ml
-	PL.7029	Methylene Blue	2000ml
-	PL.7030/100	Malachite Green	100ml
-	PL.7030/25	Malachite Green	250ml
-	PL.7030	Malachite Green	500ml
-	PL.7031	Malachite Green	1000ml
-	PL.7032	Malachite Green	2000ml

Per 100ml solution:

- Ready to use ZN Carbol Fuchsin contains 1.48g of Basic Fuchsin powder.
- Diff for ZN and Kinyoun CF contains 3ml of Hydrochloric Acid and 97ml of IMS.
- Ready to use Methylene Blue contains 0.4g of Methylene Blue powder.
- Ready to use Malachite Green contains 0.4g of Malachite Green powder.

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 deionised or reverse osmosed water before use):

-	PL.8005	ZN Carbol Fuchsin	100 ml
-	PL.8005/4.0	ZN Carbol Fuchsin	400 ml
-	PL.8005/5.0	ZN Carbol Fuchsin	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

Per 100ml solution:

- Concentrated ZN Carbol Fuchsin contains 11.8g of Basic Fuchsin powder.
- 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.
- Any serious incident that occurs in relation to the device should be reported to the manufacturer and the competent authority of the member state in which the incident occurred.

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 ZN CF 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 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 *Isospora* 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. *Centraldl. 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 Farbung des Tuberkelbacillus. *Dtsch. Med. Wochenschr.* 8:451.

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CE

	= Use by
LOT	= Lot number
REF	= Catalogue number
	= Manufacturer
EC REP	= Authorized Representative in the European Community
Σ	= 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.

*NCTC® and NCPF® are trademarks of Public Health England. ATCC® strains are listed for reference only. ATCC® is a registered trademark of the American Type Culture Collection.

HAZARDS IDENTIFICATION

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

 DANGER	PL.8007 PL.8007/4.0 PL.8007/5.0	H226, H302, H318, H332, H361, H411 P210, P273, P280, P305+P351+P338, P310, P370+P378, P391, P403+P235
 DANGER	PL.7018/100 PL.7018/25 PL.7018 PL.7019 PL.7020	H302, H314, H332, H341, H410 P260, P273, P280, P303+P361+P353, P305+P351+P338, P310, P391, P321
 DANGER	PL.8005 PL.8005/4.0 PL.8005/5.0	H301, H311, H314, H331, H341, H373, H410 P260, P273, P280, P303+P361+P353, P305+P351+P338, P310, P331, P391, P403+P233, P321
 DANGER	PL.7024/100 PL.7024/25 PL.7024 PL.7025 PL.7026	H225, H302, H311, H331, H371 P210, P260, P264, P321, P370+P378, P403+P233
	PL.8006 PL.8006/4.0 PL.8006/5.0	H301, H311, H331, H332, H370, H226 P210, P260, P301+P310, P321, P370+P378, P403+P233
 WARNING	PL.7030/100 PL.7030/25 PL.7030 PL.7031 PL.7032	H226, H319, H412 P210, P273, P337+P313, P370+P378, P403+P235, P501
 DANGER	PL.7027/100 PL.7027/25 PL.7027 PL.7028 PL.7029	H302, H332, H370 P260, P264, P270, P308+P311, P501, P321