Acid-Fast Stains for Mycobacteria (Auramine Phenol)

(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 Auramine Phenol 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. Auramine phenol tends to show a higher sensitivity and specificity than the Kinyoun or ZN methods, and therefore is generally preferred when screening suspected tuberculosis cases where the bacilli count may be low.

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 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.7033/100	Auramine Phenol	100ml
-	PL.7033/25	Auramine Phenol	250ml
-	PL.7033	Auramine Phenol	500ml
-	PL.7034	Auramine Phenol	1000ml
-	PL.7035	Auramine Phenol	2000ml
-	PL.7036/100	Auramine Differentiator	100ml
-	PL.7036/25	Auramine Differentiator	250ml
-	PL.7036	Auramine Differentiator	500ml
-	PL.7037	Auramine Differentiator	1000ml
-	PL.7038	Auramine Differentiator	2000ml
-	PL.7144	Auramine Differentiator (1% HCl)	500ml
-	PL.7145	Auramine Differentiator (1% HCl)	1000ml
-	PL.7146	Auramine Differentiator (1% HCl)	2000ml
-	PL.7039/100	Potassium Permanganate	100ml
-	PL.7039/25	Potassium Permanganate	250ml
-	PL.7039	Potassium Permanganate	500ml
-	PL.7040	Potassium Permanganate	1000ml
-	PL.7059/100	Thiazine Red	100ml
-	PL.7059/25	Thiazine Red	250ml
-	PL.7059	Thiazine Red	500ml
-	PL.7060	Thiazine Red	1000ml

Per 100ml solution:

- Ready to use Auramine Phenol contains 0.4g of Auramine O and 4g of Phenol.
- Auramine Differentiator contains 0.5ml of Hydrochloric Acid and 75ml of IMS.
- Auramine Differentiator (1% HCl) contains 1ml of Hydrochloric Acid and 99ml of IMS.
- Ready to use Potassium Permanganate contains 0.1g Potassium Permanganate powder.
- Thiazine Red contains 0.2g of Thiazine Red powder.

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

-	PL.8008	Auramine Phenol	100ml
-	PL.8008/4.0	Auramine Phenol	400ml
-	PL.8008/5.0	Auramine Phenol	500ml
-	PL.8013	Potassium Permanganate	100ml
-	PL.8013/4.0	Potassium Permanganate	400ml
-	PL.8013/5.0	Potassium Permanganate	500ml

Per 100ml solution:

- Concentrated Auramine Phenol contains 4g of Auramine O and 40g of Phenol.
- Concentrated Potassium Permanganate contains 1g Potassium Permanganate 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 15-25ºC in their original containers. Protect Potassium Permanganate from light. Products 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 Auramine Phenol, stand for 10 minutes.
4. Rinse with water.
5. Flood the slide with differentiator for 10 minutes, applying a change of differentiator at 5 minutes.
6. Rinse with water.
7. Flood the slide with Potassium Permanganate or Thiazine Red, stand for 30 seconds.
8. Rinse well with water; gently blot dry or dry using gentle heat.
9. Examine using a fluorescent 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

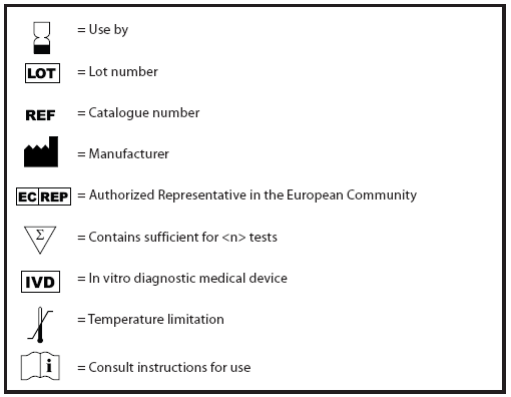
Acid-fast positive bacilli appear as bright fluorescent rods against a dark background.
Acid-fast negative bacilli will appear dark in colour. No fluorescence will be observed.

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 *Mycobacterium* 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* species 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. *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.



EC REP Advena Ltd. Tower Business Centre, 2nd Floor, Tower Street, Swatar, BKR 4013, Malta.

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Canada: Toll Free +1 800 268 2341 Tel +1 905 731 0300
20 Mural Street, Unit 4, Richmond Hill, ON, L4B 1K3
www.pro-lab.com/home/

U.S.A: Tel (512) 832-9145
21 Cypress Blvd, Suite 1155, Round Rock, Texas, 78665-1046
www.pro-lab-direct.com

UK: Tel +44 (0)151 353 1613
3 Bassendale Road, Bromborough, Wirral, CH62 3QL
www.pro-lab.co.uk

Revision: 2022 08

HAZARDS IDENTIFICATION

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

 DANGER	PL.7033/100 PL.7033/25 PL.7033 PL.7034 PL.7035	H314, H341, H412 P273, P280, P303+P361+P353, P305+P351+P338, P310, P501
 DANGER	PL.8008 PL.8008/4.0 PL.8008/5.0	H226, H301+H331, H312, H314, H341, H351, H373, H411 P210, P273, P280, P301+P330+P331, P310, P302+P352, P304+P340, P305+P351+P338, P501
 DANGER	PL.7036/100 PL.7036/25 PL.7036 PL.7037 PL.7038 PL.7144 PL.7145 PL.7146	H225, H332, H319, H371 P210, P270, P280, P303+P361+P353, P304+P340, P305+P351+P338, P312, P501
	PL.7039/100 PL.7039/25 PL.7039 PL.7040	H412 P273, P501
	PL.8013 PL.8013/4.0 PL.8013/5.0	H411 P273, P391, P501
 WARNING	PL.7059/100 PL.7059/25 PL.7059 PL.7060	H315, H319, H341 P202, P280, P302+P352, P305+P351+P338, P308+P313, P501