AURAMINE O STAIN KIT

- For in vitro use only - Catalogue No. SA85/86/87

Our Auramine O Stain is a fluorochrome stain used in the microscopic detection and examination of acid-fast mycobacteria.

Acid-fast organisms have cell walls that are resistant to conventional staining by aniline dyes such as the Gram stain. However methods that promote the uptake of dyes are available; once stained these organisms are not easily decolorized even with acid-alcohol or acid-acetone solutions and therefore they are described as acid fast. Their resistance to destaining is a useful characteristic in differentiating these organisms from contaminating organisms and host cells.

Auramine O is a non-specific fluorochrome dye with a high affinity for mycobacteria. In the case of *Mycobacterium* species the dyes can bind the mycolic acids contained in their cell walls allowing penetration of the stain. Stained mycobacteria will fluoresce yellow when examined with a fluorescent microscope, but fluorescent-positive samples should be considered preliminary until confirmed through further testing (i.e. culture techniques). Positive fluorochrome slides may be directly restained with Ziehl-Neelsen or Kinyoun Stain after removal of immersion oil with xylene. This may be done to confirm positive findings and to study the organism morphology in more detail.

Formulation per 100 mL

SA85 Auramine O Stain

Auramine O	0.1 g
Phenol	3.0 g
Ethanol	10 mL
Purified water	90 mL

SA86 Auramine Decolorizer

Hydrochloric acid	0.5 mL
Ethanol [70%]	70.0 mL

SA87 Auramine Counterstain (Potassium Permanganate)

Potassium permanganate	0.5 g
Purified water	100.0 mL

Recommended Procedure

1. Place the slide containing the fixed smear onto a level staining rack. Ensure that you have access to distilled or deionized water for the rinsing process prior to proceeding.
2. Flood the slide with Auramine O Stain. Allow the smear to stain for 15 minutes. Ensure the stain stays on the smear.
3. Rinse the slide thoroughly with distilled water and shake off any excess fluid.
4. Flood the stain with the Auramine Decolorizer. Allow the smear to decolorize for 2 minutes.
5. Rinse the slide thoroughly with distilled water and shake off any excess fluid.
6. Flood the slide with Auramine Counterstain. Allow the smear to stain for 2 minutes. (Do not exceed the 2 minute mark as the counterstain may quench the intensity of fluorescence observed)
7. Rinse thoroughly with distilled water and allow smear to air dry. Do not blot.
8. Examine microscopically using a fluorescent microscope as soon as possible. Use a 20x or 40x objective for screening, and a 100x oil immersion objective to observe the morphology of fluorescing organisms.
9. If desired, the slide can be directly restained using one of the other acid-fast stains (Ziehl-Neelsen or Kinyoun Stain) after the immersion oil is removed.

Interpretation of Results

Auramine O stain will bind to mycobacteria, which appear as bright yellowish-green, luminous rods against a dark background. Mycobacteria are

approximately 1 to 10-µm long and typically appear as slender rods. However they may also appear curved or bent, coccobacillary or filamentous. Some may be beaded or banded.

Other acid fast organisms may also be stained by auramine O; microorganisms showing degrees of acid fastness include *Nocardia*, *Rhodococcus*, *Legionella micdadei*, cysts of *Cryptosporidium* species, and *Cyclospora* species.

Non-acid-fast organisms including host tissue cells will stain dark violet or black and will not fluoresce.

- *Avoid excessive treatment with counterstain as a longer staining time may quench the fluorescence of acid-fast organisms*
- *If slides can not be read immediately store slides in a dark place at 4 to 8℃*
- *Rapidly growing mycobacteria may vary in their ability to retain acid-fast dyes and may fail to fluoresce using auramine stain*
- *Be aware of adequate safety precautions and procedures required when handling specimens that are submitted for mycobacterial evaluation. Since heat fixing and staining may not kill all mycobacteria, discard slides in a sharp receptacle and wear gloves*
- *Culture techniques are much more sensitive than all acid-fast staining procedures*

Quality Control

It is recommended that a control slide be included with each run of stains. This will verify the correct performance of the procedure as well as the staining intensity of the acid-fast organisms.

Organism	Expected Result
Mycobacterium tuberculosis ATCC 25177 (H37Ra)	Yellowish-green rods fluorescence
Escherichia coli ATCC 25922	No fluorescence

Storage and Shelf life

Our Auramine O Stain, Decolorizer and Counterstain should be stored at room temperature and protected from light. Under these conditions the stain and decolorizer have a shelf life of 52 weeks from the date of manufacture, while the counterstain has a shelf life of 26 weeks from the date of manufacture.

References

1. Truant JP, Brett WA, Thomas W Jr. Fluorescence microscopy of tubercule bacillus stained with auramine and rhodamine. Henry Ford Hosp Med Bull 1962; 10:287.
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3. Baron EJ, Finegold SM. Bailey and Scott's diagnostic microbiology. 8th ed. St. Louis: Mosby, 1990.
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5. Salfinger M, Pfyffer HE. The new diagnostic Mycobacteriology laboratory. Eur J Clin Microbiol Infect Dis 1994; 13:961-79.
6. Murray PR, Baron E, Pfaller M, Tenover F, Yolken. Manual of clinical microbiology. 7th ed. Washington: ASM, 1999.

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