# MODIFIED AURAMINE-RHODAMINE STAIN KIT

- For in vitro use only - Catalogue No. SA91K/92/93/94

Our Modified Auramine-Rhodamine Stain is a fluorochrome stain used in the microscopic examination of acid-fast microorganisms such as *Mycobacterium*, *Cryptosporidium*, and *Isospora*.

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 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 and rhodamine are non-specific fluorochrome dyes that have an affinity for acid fast organisms. In the case of *Mycobacterium* the dyes can bind the mycolic acids contained in the cell wall allowing penetration of the stain. All acid fast organisms, including coccidia and sporozoan parasites, can be stained using the Modified Auramine-Rhodamine Stain. Stained acid-fast cells and cysts will fluoresce when examined with a fluorescent microscope, but fluorescent-positive samples should be considered preliminary until confirmed through further testing. 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**

SA92 Modified Auramine-Rhodamine Stain

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|---|---|
| Auramine O | 0.28 g |
| Rhodamine | 0.14 g |
| Phenol | 17.0 mL |
| Ethanol | 23.5 mL |
| Glycerin | 19.0 mL |
| De-ionized Water | 40.5 mL |

SA93 Modified A-R Decolorizer

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|---|---|
| Hydrochloric Acid | 0.5 mL |
| Ethanol | 70.0 mL |
| De-ionized Water | 29.5 mL |

SA94 Modified A-R Counterstain

| | |
|---|---|
| Malachite Green | 1.0 g |
| De-ionized Water | 100.0 mL |

**Recommended Procedure**

1. Place Modified Auramine-Rhodamine Stain, Decolorizer, and Counterstain in separate Coplin Jars. (Solutions should be changed every 30 to 40 slides)
2. A loopful of the specimen should be smeared onto a clean glass slide. Allow slide to air dry.
3. Place slide into methanol for 1 to 2 minutes to fix the specimen onto the slide. Allow slide to air dry.
4. Place slide in Modified Auramine-Rhodamine Stain for 5 minutes.
5. Rinse the slide thoroughly with distilled water and shake off excess fluid.
6. Dip slide in the Decolorizer solution for 30 seconds.
7. Rinse the slide thoroughly with distilled water and shake off excess fluid.
8. Dip slide in the Modified A-R Counterstain for 10 to 30 seconds.
9. Rinse thoroughly with distilled water and allow to air dry. Do not blot.
10. 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.
11. If desired, the slide can be directly restained using one of the other acid-fast stains (Ziehl-Neelsen or Kinyoun Stain).

**Interpretation of Results**

Properly stained acid-fast cells and cysts will fluoresce yellow or orange (color may vary with the filter system used) against a dark background when examined under a fluorescent microscope.

Non-acid-fast organisms including host tissue cells will stain green 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-Rhodamine Stains*
- *To minimize pathogenicity of samples, specimens suspected of containing Cryptosporidium should be collected and preserved in 10% formalin or SAF fixative since Cryptosporidium oocysts are immediately infectious on passage*
- *Be aware of adequate safety precautions and procedures required when handling specimens that are submitted for mycobacterial evaluation*
- *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) | Yellow to orange fluorescence |
| *Escherichia coli* ATCC 25922 | No fluorescence |

**Storage and Shelf life**

Our Modified Auramine-Rhodamine Stain, Decolorizer and Counterstain should be stored at room temperature and protected from light. Under these conditions they have a shelf life of 52 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.
4. Isenberg HD, Ed. Clinical microbiology procedures handbook, Vol 1. Washington DC: ASM, 1992.
5. Murray PR, Baron E, Pfaller M, Tenover F, Yolken. Manual of clinical microbiology. 7th ed. Washington: ASM, 1999.



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