

AFB AURAMINE-RHODAMINE TM KIT

Kit Components

- 6510-08 AFB Auramine-Rhodamine TM Kit (3x8oz bottles)
 - 1x 6560-08 AFB Auramine-Rhodamine (8oz)
 - 1x 6562-08 AFB Decolorizer (Truant-Moore) (8oz)
 - 1x 6563-08 AFB Potassium Permanganate 0.5% (8oz)
- 6560 AFB Auramine-Rhodamine Replacement Kit (4x8oz bottles)
 - 4x 6560-08 Auramine-Rhodamine (8oz)
- 6562 AFB Decolorizer (Truant-Moore) Replacement Kit (4x8oz bottles)
 - 4x 6562-08 AFB Decolorizer (Truant-Moore) (8oz)
- 6563 AFB Potassium Permanganate 0.5% Replacement Kit (8oz)
 - 4x 6563-08 AFB Potassium Permanganate 0.5% (8oz)

Intended Use

Recommended for use in qualitative and quantitative procedures in the fluorescent microscopic detection of mycobacteria.

Principle and Results

The use of fluorescent dyes for the detection of acid-fast bacilli in clinical specimens was described by Hagemann in 1937.¹ In 1962, Truant, Brett, and Thomas evaluated these methods usefulness and found that the fluorescent staining technique yields a larger number of positive smears compared to fuchsin-stained method.² Auramine-rhodamine, a fluorochrome dye, forms a complex with mycolic acids found in the acid-fast organism's cell walls. This complex resists decolorization by acid-alcohol. Potassium permanganate, a strong oxidizing agent, acts as a quenching agent, decreasing the background fluorescence of cellular debris.³ Acid-fast organisms will stain yellow-gold to yellow-orange with a black background under a fluorescent microscope.

Specimen Collection and Storage

Store products at 15-30°C, away from direct light. Liquid Media: Add a drop of suspension onto a clean glass slide using a sterile transfer pipette. Allow air drying before fixing.

Solid Media: Place a drop of saline on a clean glass slide. Using a sterile loop, pick up a small colony and transfer to the saline drop. Spread the suspension onto slide and allow air drying before fixing. Heat-fix air dried smears at 80°C for at least 15 minutes; or for 2 hours on an electric hot plate at 65-70°C.⁴ Or methanol fix by submerging slide(s) into methanol for a minimum of 30 seconds and allowing it to dry before staining.

Precautions

For use by laboratory professionals. See SDS for complete warnings, precautions, hazard and precautionary statements, and disposal information.

Quality Control

Ethos Biosciences tests each lot using appropriate microorganisms following CLSI document M22-A3 *Quality Assurance of Commercially Prepared Microbiological Culture Media*.

It is recommended that end-users perform their own quality control suggested by their facility. Organisms used in testing: *Mycobacterium* spp. for positive and a non-acid fast organism mixture for negative.

Results

Positive bacteria	Yellow-gold to yellow-orange
Negative bacteria	Not visible/black

Limitations

1. Positive staining provides presumptive evidence of the presence of mycobacteria. Negative staining does not indicate that the culture will be negative. A culture must be performed with staining.
2. Rapid grower may not appear fluorescent. It is recommended that all negative smears be confirmed with fuchsin staining.
3. Stained smears should be observed within 24 hours after staining due to fluorescence potentially fading.
4. Tap water is not recommended when making smears or performing rinsing. *M. gordonae* has been found in tap water and may interfere with assessment.⁴
5. Organisms grown in media will typically stain better. Organisms grown on blood agar can limit acid-fast properties in staining.⁴

References

1. Hagemann PKH. Fluoreszenzmikroskopische Untersuchungen über Virus und andere Mikroben. *Zentralblatt für Bakteriologie*. 1937;140:184-187.
2. Truant JP, Brett WA, Thomas W, Jr. Fluorescence microscopy of tubercle bacilli stained with auramine and rhodamine. *Henry Ford Hosp Med Bull*. Jun 1962;10:287-96.
3. Madison BM. Application of stains in clinical microbiology. *Biotech Histochem*. May 2001;76(3):119-25.
4. Hussey MA, Zayaitz A. Acid-Fast Stain Protocol. *American Society of Microbiology*; 2008;2025(July 22):1-12. <https://asm.org/protocols/acid-fast-stain-protocols>

PROCEDURE						
#	ACTION	SOLUTION	HEAT	TIME		DETAILS
				MIN	SEC	
1	FLOOD	AURAMINE-RHODAMINE	–	15	–	FLOOD OR SUBMERGE SLIDE
2	RINSE	RUNNING WATER	–	–	–	RINSE TILL WATER RUNS CLEAR
3	FLOOD	ACID DECOLORIZER	–	2-3	–	FLOOD OR SUBMERGE SLIDE
4	RINSE	RUNNING WATER	–	–	–	RINSE UNTIL WATER RUNS CLEAR
5	RINSE	POTASSIUM PERMANGANATE	–	4	–	FLOOD OR SUBMERGE SLIDE
6	RINSE	RUNNING WATER	–	–	–	
7	DRY		–	–	–	ALLOW SLIDE TO DRY BEFORE USING IMMERSION OIL

PRODUCT INFORMATION

CAT. #	DESCRIPTION
6510-08	AFB Auramine-Rhodamine TM Kit (3x8oz bottles)
6560	AFB Auramine-Rhodamine Replacement Kit (4x8oz bottles)
6562	AFB Decolorizer (Truant-Moore) Replacement Kit (4x8oz bottles)
6563	AFB Potassium Permanganate 0.5% Replacement Kit (8oz)

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