# HiFluo-Phenol Free Stain Kit K061

## Intended use

HiFluo – Phenol free Stain Kit is recommended for fuorescent staining of Acid-Fast-Bacteria (Mycobacterium).

## Kit Contents

| | |
|---|---|
| S082 Auramine-Rhodamine Solution (Phenol free) | 200ml |
| S099 Decolourizer | 2X200ml |
| S083 Potassium Permanganate Solution | 200ml |

## Composition**

| **Ingredients** | |
|---|---|
| **Auramine-Rhodamine Solution (Phenol free)(S082)** | - |
| Auramine O | 12.0 g |
| Glycerine | 600.0 g |
| Rhodamine B | 6.0 g |
| Phenyl ethyl alcohol | 85.0 ml |
| 2-Proapnol | 140.0 ml |
| Distilled water | 157.0 ml |
| **Decolourizer (S099)** | - |
| Hydrochloric acid,concentrated | 3.0 ml |
| Ethyl alcohol,95% | 97.0 ml |
| **Potassium Permanganate Solution (S083)** | - |
| Potassium permanganate | 5.0 g |
| Distilled water | 1,000.0 ml |

**Formula adjusted, standardized to suit performance parameters

## Directions

1. Fix smears on electric slide warmer at 65-75 BC for at least 2 hours, or use a Bunsen Burner. Do not overheat.
2. Flood smears with Auramine-Rhodamine solution (phenol free) (S082) and allow to stain for 15 minutes, making certain that the staining solution remains on the smear. Do not apply heat to smear. Do not use filter strips.
3. Rinse smears with chlorine free water for 30 seconds and drain.
4. Flood smears with decolourizer (S099) and allow de-staining for 1 minutes.
5. Rinse again with chlorine free water for 30 seconds and drain.
6. Counter stain with potassium permanganate solution (S083) for 2-5 min. Do not allow slide to dry.
7. Rinse again with chlorine free water for 30 seconds and drain.
8. Allow the smear to air dry and observe under fluorescent microscope.

## Principle And Interpretation

HiFluo Phenol free Stain Kit uses auramine-rhodamine stain (AR), also known as the Truant auramine-rhodamine stain. It

is a histological technique used to visualize acid-fast bacilli using fluorescence microscopy, notably species in the Mycobacterium genus. The HiFluo Phenol free Stain is a modification of Truant et.al. methods which, makes the inclusion of phenol in staining solution unnecessary. The sensitivity and specificity of the staining results are identical with those obtained using the classical method employing Phenol. Auramine and Rhodamine are nonspecific fluorochrome dyes that have an affinity for acid fast organisms. In case of Mycobacteria, the dyes can bind specifically to the mycolic acid contained in the cell wall allowing the penetration of the stain.

This complex resists decolorization by acid-alcohol decolorizer solution. The counterstain, potassium permanganate helps to prevent nonspecific fluorescence, thus reducing the possibility of artifacts. When observed under the microscope with UV illumination, acid fast cells are yellow or bright orange against dark background.

## Type of specimen

Any isolated colony on primary or subculture plates can be isolated from following specimens. Clinical specimen: Blood, urine, CSF, pus, wounds, lesions, body tissues, sputum etc.

## Specimen Collection and Handling

All testing for acid-fast bacilli is sent to the reference laboratory in an effort to meet the 24hr TAT time for smear results. Use sterile, leak proof disposable plastic containers for collection. Do not use wax containers as these can cause false positive smear results. Do not use any fixative or preservatives. Swabs are not recommended as a collection device for the isolation of mycobacteria. They are acceptable only if the specimen cannot be obtained by any other means. A negative result from a swab specimen is not reliable. In general, the number of acid fast bacilli in a specimen is small. Early morning specimens are the specimens of choice for urine and sputum because the mycobacteria have had a chance to pool and concentrate, and so increase the chances of recovery. Always collect and submit the maximum volume possible of specimens normally considered sterile. Do not submit 24-hour collections, as they are likely to be diluted and contaminated.

Collect specimens before antimicrobial therapy is started. Even a few days of therapy may kill or inhibit sufficient numbers of mycobacteria to prevent recovery on culture and so leave confirmation of disease in doubt. If a specimen is submitted after therapy has been initiated, note on the request. Avoid contamination of the specimen with tap water, as environmental mycobacteria exist and their recovery by smear or culture can cause confusion for the patient diagnosis.

## Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations

1. Smear that are too thick may flake during staining and may be difficult to decolorize.
2. Chlorine in water may interfere with fluorescence since use of chlorine free water for in between washing is mandatory.
3. Time is critical with potassium permanganate because counter staining for a longer time may quench fluorescence of acid-fast bacilli.

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## Quality Control

- **Microscopic examination :** Acid fast staining is carried out and characteristic staining is observed under fluorescent microscope
- **Results :** Acid-Fast Bacteria: Depending on the filter combination used, Acid Fast Bacteria show red-orange or yellow green fluorescence.
  Back ground: Dark background, debris may display light yellow fluorescence.

## Storage and Shelf Life

Store between 10-30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques

## Reference

1. Murray, P.R. (Editor in chief), (1999). Manual of clinical Microbiology. 7th ed. American society for Microbiology, Washington, D.C. Truant, J.P., Brett, W.A. and Thomas, W. (1962). Fluorescent microscopy of tubercle bacilli stained with auramine and Rhodamine. Henry Ford Hospital Bulletin, 10, 287-96.
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4. Shanhooltzer, C.J., P. Schaper ,and L.R. Peterson. 1982. Concentrated Gram stain smear prepared with a cytospin centrifuge. J. clin. Microbiol.16:1052-1056
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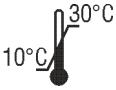

Storage temperature


Do not use if package is damaged

In vitro diagnostic medical device


CE Marking


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