HiCold Stain TB Kit- Kit for Mycobacteria

HiCold Stain TB Kit is recommended for microscopic investigation of *Mycobacterium* by Cold acid-fast staining method (Kinyoun method)

**Composition**

**Ingredients**

**Carbol fuchsin solution (S080)**

- Basic fuchsin: 4.0 gm
- Ethyl alcohol, 95%: 25.0 ml
- Phenol: 10.0 ml

**Decolorizer (S099)**

- Conc. HCl: 3.0 ml
- Ethyl alcohol, 95%: 97.0 ml

**Counter stain (Loeffler’s methylene blue) (S081)**

- Methylene blue chloride: 0.3 gm
- Ethyl alcohol, 95%: 30.0 ml
- KOH solution (0.1% aqueous): 100.0 ml

**Formula adjusted, standardized to suit performance parameters**

**Directions**

**Pretreatment of sputum:**

Aseptically mix 0.1 gm of N-Acetyl-L-Cysteine (R034) in 20 ml of Sodium citrate-hydroxide buffer (R032). Use immediately and within 24 hours only. Transfer a maximum volume of 10 ml of sputum/clinical sample to a sterile graduated 50 ml plastic centrifuge tube having a leak proof cap. Add an equal volume of the above solution. Mix the contents thoroughly by inverting with the cap tightened. Mix on vortex mixer for approximately 20s until the contents are liquefied. Allow the mixture to stand for 15 minutes at 20 to 25°C with occasional gentle shaking by hand. Do not overprocess as this will reduce the recovery of mycobacteria. Add phosphate buffer (R033) up to the 50 ml mark on the tube. Recap the tube and swirl it by hand to mix the contents well. Centrifuge the solution for at least 15 minutes at >= 3000 rpm. Preferably use a refrigerated centrifuge. Carefully decant the supernatant fluid into a splash-proof discard container containing suitable disinfectant. Smear out the sediment and allow to dry.

**A. Smear Staining:**

1. Flood the fixed smear with the carbol fuchsin solution (S080) and wait for 15 min without heating.
2. Wash the smear with running tap water until no further colour is given off.
3. Pour decolourizing reagent (S099) on the slide and allow to stand for 30 sec for thin smear and upto 2 min for thick smear.
4. Wash immediately with tap water.
5. Counter stain for 1-2 min with Loeffler’s methylene blue (S081).
6. Rinse well with tap water and dry.
7. Observe under low power objective and examine under oil immersion objective.

**B. Tissue (histological section) staining:**

1. Deparaffinize sections in 2 changes of Xylene, and absolute alcohol. Air dry slides.
2. Flood the slide with carbol fuchsin solution (S080), and allow standing at 37°C for 1 hour or at 56°C for 30 min.
Mycobacteria (AFB/Acid Fast Bacteria) are difficult to stain due to high lipid and wax content in their cell walls. HiCold TB stain kit is modified Ziehl-Neelsen staining method which does not require heating (Kinyoun method). The omission of heating step is made possible by increasing the concentration of basic fuchsin in carbol fuchsin solution. When stained with strong stains (carbol fuchsin), acid acid-fast bacilli retain their colour even after treatment with strong decolourizing solutions. They remain red after counterstaining with loeffler’s methylene blue, whereas the microorganisms susceptible to acid take on the blue colour.

**Principle And Interpretation**

Mycobacteria (AFB/Acid Fast Bacteria) are difficult to stain due to high lipid and wax content in their cell walls. HiCold TB stain kit is modified Ziehl-Neelsen staining method which does not require heating (Kinyoun method). The omission of heating step is made possible by increasing the concentration of basic fuchsin in carbol fuchsin solution. When stained with strong stains (carbol fuchsin), acid acid-fast bacilli retain their colour even after treatment with strong decolourizing solutions. They remain red after counterstaining with loeffler’s methylene blue, whereas the microorganisms susceptible to acid take on the blue colour.

**Quality Control**

**Microscopic examination**

Cold acid fast staining is carried out and staining characteristics is observed under microscope using oil immersion lense.

**Results**

- Acid fast Bacteria : Bright Red bacilli
- Other type Bacteria : Blue
- Other tissue element : Blue

**Storage and Shelf Life**

Store below 30°C in tightly closed container and away from bright light. Use before expiry date on label.

**Reference**

2. George Clark; Staining procedures, fourth edition. Published by Williams and Wilkins, Baltimore. 1981.

**Important Note:**

1. This kit is for ‘in-vitro’ diagnostic use only.
2. Upon completion of work keep the all the reagent bottles tightly closed, away from bright light, under recommended storage conditions.
3. Staining must be carried out by qualified personnel. National guidelines for work and safety must be followed.
4. The clinical interpretation of result (positive or negative), should be carried out by a qualified pathologist. The interpretation should be complemented by morphological studies and appropriate controls. It is further advised to evaluate the staining results with patient’s clinical history, symptoms and parallel diagnostic test, for confirmation.
5. Used and expired solutions must be disposed as special waste in accordance with local guide lines.