MB545D **HiPurA® Decontamination Kit for MTB**

Kit Contents

Product Code	Reagents provided	20 Preps
DS0216	4% NaOH	120 ml
DS0217	N-Acetyl-L-cysteine	0.1g x 20 nos.
DS0218	Sodium citrate-hydroxide buffer	20 ml x 20 nos.
DS0219	Phosphate buffer	100 ml x 20 nos.

Intended Use

Recommended for decontamination of other organisms from human sputum, cells and tissue samples.

Introduction

The diagnosis, treatment and monitoring of tuberculosis are conducted in a wide range of laboratory facilities worldwide, using a variety of methods, equipments and capacity. The recovery of Mycobacteria from sputum or other samples like CSF or tissue specimens contaminated with other organisms is difficult, since mycobacteria generally grow much slower than other bacterial species. Decontamination and digestion of the mucous components kills contaminating normal flora and allows slower growing mycobacteria to grow. HiPurA® Decontamination Kit for MTB is used to break down mucous components of sputum and to decontaminate the specimen of normal flora in order to allow slower growing mycobacteria to grow**.**

Materials needed but not provide:

- Sterile Mortar & Pestle
- 50ml Centrifuge tubes
- Tissue Homogenizer
- 37°C Incubator
- Tabletop centrifuge (with rotor for 50 ml tubes)
- Vortex mixer
- Sterile distilled water
- 5% phenol solution

Storage

Store the HiPurA® Decontamination Kit for MTB between 15-25°C (on receipt). Under recommended condition kit is stable for 1 year.

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED
CE

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Specimen Handling and Collection

Collect sputum sample in sterile container (if to be used for future) and store the samples at 2-8°C for short term storage or -20°C for long term storage. Ensure that the sputum sample is at room temperature (15-25°C) before beginning the protocol. After use, contaminated material must be sterilized by autoclaving before discarding.

Types of Specimen

Clinical samples: sputum, cells and tissue sample

Procedure

1. For Tissue Samples:

i. Cut the tissue sample into small pieces in a sterile mortar (or homogenizer/tissue grinder) using a clean, sterile pair of forceps and scissors.

ii. Add approximately 2ml of sterile Phosphate buffer (DS0219). Gently grind tissue in Phosphate buffer with a mortar and pestle (or homogenizer/tissue grinder) until a homogeneous suspension is obtained and continue with Protocol B.

2. For Sputum/CSF Samples:

I) Protocol A (for Non-Viscous sputum samples):

i. Transfer the sample (approximately 5 ml) to sterile centrifuge tube and add double the volume of sterile 4% NaOH solution (DS0216), aseptically.

For example: Add 20 ml of 4% NaOH solution to 10 ml sample.

ii. Tighten the caps of the tube and mix it well. Invert each tube to ensure that NaOH solution contacts all the sides and inner portion of caps.

iii. Place the tube in 37°C incubator for 15 minutes.

iv. Remove the tubes from the incubator and add 15ml of sterile distilled water.

v. Mix it well and centrifuge at 3000 x g (5000 rpm) for 15 minutes. Discard the supernatant slowly into a container with 5% phenol solution.

vi. Wash the pellet with sterile distilled water and centrifuge at 3000xg (5000 rpm) for 15 minutes and decant the supernatant.

vii. Resuspend the pellet in 500µl Sterile distilled water and store at -80°C for later use. The pellet can be used for DNA extraction protocol (HiPurA® Mycobacterium tuberculosis DNA Purification Kit-MB545).

II) Protocol B (for Viscous samples):

i) Aseptically mix 0.1 g of N-Acetyl-L-cysteine (DS0217) in 20 ml of Sodium citrate-hydroxide buffer (DS0218). Use immediately and within 24 hours only.

ii) Transfer a maximum volume of 10 ml of specimen to a sterile graduated 50 ml centrifuge tube. Add an equal volume of (N-Acetyl-L-cysteine + Sodium citrate-hydroxide buffer) above solution and mix the contents thoroughly by inverting with the cap tightened.

NOTE: If transferring the sample to a centrifuge tube is difficult due to its viscosity, then directly add the mix to the vial containing the sample and mix it well.

iii) Mix on vortex mixer for approximately 20 seconds until the contents are liquefied. Allow the mixture to stand for 15 minutes at 15-25°C with occasional gentle shaking by hand. Do not over process as this will reduce the recovery of *Mycobacterium*.

iv) Add Phosphate buffer (DS0219) 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 x g (5000 rpm) at 4°C.

v) Carefully decant the supernatant. To the sediment, add 1 to 2 ml of phosphate buffer (DS0219), pH 6.8 with a sterile pipette and resuspend the sediment with the pipette or by gently shaking the tube.

vi) Inoculate onto solid culture media (M162-LJ Medium Base, M199-Middlebrook 7H10 Agar etc.) or use for preparing a smear for staining or can be further used for DNA extraction protocol (by using HiPurA® Mycobacterium tuberculosis DNA Purification Kit [Product code: MB545]).

Warning and Precautions

Certified for *in vitro* Diagnostic Use (IVD). Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Limitations

1. This test has to be confirmed by streaking the sample on nutrient agar plates for the growth of other organisms.

Performance and Evaluation

Performance of the kit is expected when the kit is used as per the protocol mentioned in the product insert within the expiry period when stored at recommended temperature.

Quality Control

Type of Sample	Growth of other bacteria
Sputum sample	None

Safety Information

The HiPurA® Decontamination Kit for MTB is for laboratory use only, not for drug, household or other uses. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

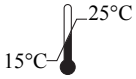
Please refer disclaimer Overleaf.

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 off in accordance with current laboratory techniques.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail to mb@himedialabs.com

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
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