TiBO - Trends In Innovative Biotechnology Organization

MYCOPROSAFE®

DECONTAMINATION AND CONCENTRATION KIT
FOR MYCOBACTERIAL CULTURE, MICROSCOPY AND MOLECULAR METHODS

Cat. #: MPS220, MPS230, MPS240 **Instructions for Use** **For In Vitro Diagnostic Use**

Product's name:

MYCOPROSAFE® (MPS®)

Product's intended use:

MPS® is a sample decontamination and concentration kit. It permits the processing of samples for microscopy, culture and molecular methods for identification and isolation of mycobacteria. It is intended for **in vitro diagnostic use**.

General information:

MPS® is a kit that contains all the materials needed to safely perform the "sodium hydroxide - N-acetyl-L-cysteine (NALC) decontamination and concentration method".[1,2,3] This method is recommended to increase efficiency in the recovery of mycobacteria from samples by selectively killing other microorganisms and liquefying samples like sputum for better sedimentation. Currently, materials needed for this purpose are either prepared by the laboratories that process the samples, or are purchased. In any case, the buffers used in this procedure are stored in big containers and they are a prime source of contamination, since they themselves are frequently contaminated during multiple use. ***MPS®*** provides, in individual sets, all the materials needed for processing each sample. It eliminates the problem of cross-contamination while saving time and effort. It is designed, to be disposed of safely, after processing biohazard material.

Limitations of the method:

Some organisms in samples other than mycobacteria, may survive the decontamination procedure.

MPS® is intended as a general-purpose device. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic). It is the user's responsibility to validate the performance of ***MPS®*** for any particular use, since its performance characteristics have not been validated for any specific organism. ***MPS®*** may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.

Principles of the procedure:

Clinical samples like sputum contain many microorganisms other than mycobacteria. Processing with sodium hydroxide - sodium citrate - NALC, decontaminates the samples by killing many microorganisms susceptible to sodium hydroxide while mycobacteria, that are resistant to alkaline pH, survive. NALC is a reducing substance that reduces the disulfide bonds of the mucus proteins which decreases viscosity of the sputum and thus liquefies the samples. This facilitates the sedimentation of bacilli during centrifugation.

Ingredients:

One box of ***MPS®*** contains:

- 36 units of N-Acetyl-L-cysteine (NALC), ≈ 40 mg, 100%, in 50 mL plastic tube.
- 36 units of sodium hydroxide (2%, 3% and 4% in MPS220, MPS230 and MPS240, respectively), trisodium citrate·$3H_2O$ 1.47% solution and a pH indicator, 10 mL, in plastic tube.
- 36 units of 0.067 (1/15) M sterile phosphate buffer (Na_2HPO_4 + KH_2PO_4), pH = 6.8 - 7.0, 50 mL, in plastic bottle.

Cautions and warnings:

FOR IN VITRO DIAGNOSTIC USE.

Laboratory procedures involving mycobacteria require special equipment and techniques to minimize biohazards. Specimen preparation must be done in a biological safety cabinet.

MPS® has been designed to minimize risks associated with mycobacterial testing. However to further reduce the risks of accidental exposure to infectious agents, additional precautions should be taken. At a minimum, specimen manipulation should be done in a contained environment with controlled access, which has a tuberculosis exposure control plan. The locations should have surfaces, which can be easily decontaminated using an appropriate topical disinfectant. Pathogenic microorganisms including Hepatitis B Virus and Human Immunodeficiency Virus (HIV) may be present in specimens. Universal precautions and local laboratory guidelines should be followed in handling all items contaminated with blood or body fluids. If a tube is found to be leaking or is accidentally broken during collection or transport, use the established procedures in your facility for dealing with mycobacterial spills. At a minimum, universal precautions should be employed.

General safety precautions:

- Always wear masks and gloves when working with potential biohazard material.
- Work in a laminar flow cabinet, biosafety level II, when pipetting the samples.
- Never use mouth pipetting.
- A refrigerated centrifuge with airtight swinging buckets is recommended for sedimenting bacteria to minimize aerosols.
- Use only conical centrifuge tube holders of 30 mm of diameter adapted to the shape of the sampling tubes.
- If spills of the contaminated material occur, disinfect with 2.5% hypo-chloride solution.
- If sodium hydroxide - sodium citrate solution or disinfectant solution contacts skin, eyes or mucosal surfaces, wash immediately and thoroughly with water and seek immediate medical help.
- Do not give any part of the kit to children.
- Pathogenic microorganisms including Hepatitis B virus and Human Immunodeficiency Virus (HIV) may be present in specimens. Universal precautions and local laboratory guidelines should be followed in handling all items contaminated with blood or other body fluids. If a tube is found to be leaking or is accidentally broken during collection or transport, use the established procedures in your facility for dealing with mycobacterial spills.

- At a minimum, universal precautions should be employed.
- Tubes should be discarded in an appropriate manner.

Storage instructions:

Store at room temperature, in a dry place.

Indications of instability or deterioration:

MPS® kit should not be used if above indicated volumes are not present in each tube and if there is turbidity or sediments in the phosphate buffer solution. Sediments may sometimes form in sodium hydroxide - sodium citrate. This does not alter the normal function of the kit.

List of materials provided:

List of materials for processing one sample:

- 50 mL polypropylene tube containing NALC powder.
- 10 mL sodium hydroxide - trisodium citrate solution with pH indicator in plastic tube.
- 50 mL sterile phosphate buffer in polypropylene bottle.

Each cardboard box contains 36 sets of the materials listed above.

Instructions for use[2]:

Sputum and samples other than urine:

1- Transfer a maximum volume of 10 mL of samples like sputum, bronchoalveolar fluid, gastric lavage fluid, pleural, pericardial, or peritoneal fluids from the collection cup into the tube containing NALC powder.
2- Add sodium hydroxide - sodium citrate – pH indicator solution in a quantity approximately equal to that of the sample. Close the cap tightly.
3- Homogenize the sample by shaking with a vortex and let it stand at room temperature for 15 to 30 minutes according to the contamination rates.
4- Fill the tube to the “50 mL” line with phosphate buffer solution (save the left over buffer for further steps).
5- Spin the tubes for 15 minutes at 2000 x g (use a refrigerated centrifuge with airtight buckets if available to minimize aerosol formation and killing of mycobacteria by heat produced in non-refrigerated centrifuges).
6- Carefully discard the supernatant into a container with disinfectant solution. Leave as little solution as possible on the sediment.
7- Add a few milliliters of the remaining phosphate buffer. Resuspend the sediment by vortexing. The suspension should be clear or very light orange to pink in color indicating the NaOH is neutralized with phosphate buffer and the pH is properly adjusted. This suspension can now be used for culture, microscopy and molecular diagnostic methods. If prominent pink to purple color persists, this indicates that the pH is not properly neutralized. In this case the tube can be centrifuged once more; after discarding the supernatant the sediment should be suspended with phosphate buffer.
8- Once the procedure is complete, dispose the tubes safely.

Urine samples:

It is recommended to obtain early morning urine to increase the chance of recovery of mycobacteria.

1- Transfer the urine samples from the collection cup into the sample tube that contains NALC. The tube can be filled up to the 50 mL line.
2- Spin the tubes for 15 minutes at 2000 x g (use a refrigerated centrifuge with airtight buckets if available to minimize aerosol formation and killing of mycobacteria by heat produced in non-refrigerated centrifuges).
3- Discard the supernatant according to the safety rules of your laboratory. Add approximately 3 mL of sodium hydroxide - sodium citrate – pH indicator solution onto the sediment. Close the cap securely.
4- Continue processing, by starting from step 3, described above for other types of samples.

List of materials that are not provided:

- Refrigerated centrifuge for 50 mL tubes
- Vortex
- Automatic pipettors
- Sterile pipette tips

Quality control:

Positive control: Respiratory secretions spiked with mycobacteria.

Negative control: Respiratory secretions spiked with *Escherichia coli* and *Staphylococcus aureus*.

Description of the amounts of reagents necessary, and the parameters of time and temperature:

The only reagents required are those included in the kit. The whole procedure takes 40 to 50 minutes. The procedure is performed at room temperature. It is recommended to spin in a centrifuge at 4°C to minimize aerosol formation and killing of mycobacteria by the heat produced in non-refrigerated centrifuges.

Time restrictions:

Decontamination time must be from 15 to 30 minutes. Extension of decontamination time may kill mycobacteria.

Limitations of the procedure:

The pH of the processed sample may be too high if the supernatant is not completely eliminated after spinning with the phosphate buffer. This may inhibit the growth of mycobacteria in culture media.

Bibliography:

1- Kubica GP, Dye WE, Cohn ML, Middlebrook G. Sputum digestion and decontamination with N-acetyl-L-cysteine-sodium hydroxide for culture of mycobacteria. 1963. Am. Rev. Respir. Dis. 87:775-779.
2- N-acetyl-L-cysteine-sodium hydroxide method for liquefaction and decontamination of specimens. Bailey & Scott’s Diagnostic Microbiology, Ninth Edition. Mosby-Year Book Inc. St. Louis, MO. USA. 1994, p:600.
3- Heifets LB, Good RC. Current laboratory methods for the diagnosis of tuberculosis. In “Tuberculosis” Ed. Bloom BR. ASM Press, Washington D.C. 1994. 85-110.

Manufacturer:

TiBO
Trends In Innovative Biotechnology Organization
Tuzla 34959
Istanbul/Turkey
Tel: +90 538 442 0407

Catalogue number:

MPS220, MPS230, MPS240

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