*TIBO*
*Trends In Innovative Biotechnology Organization*

# DECOCENT®

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

**Cat. #:** DCC010, DCC050, DCC030 **Instructions for Use** **For In Vitro Diagnostic Use**

***Product's name:***
***DECOCENT®***

***Product's intended use:***
***DECOCENT®*** 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:***
***DECOCENT®*** is a kit that enables to apply an improved version of Kubica ("sodium hydroxide - N-acetyl-L-cysteine decontamination and concentration) method".[1,2,3]It is designed to eliminate the disadvantages of this method. Thanks to its pH indicator and its original neutralization buffer, it allows better and easy adjustment of the final pH of the processed sample.***DECOCENT®*** provides,all the materials needed for processing the samples. It eliminates the problem of cross-contamination while saving time and effort.

Limitations of the method:
Some organisms in samples other than mycobacteria, may survive the decontamination procedure.

***DECOCENT®*** 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 ***DECOCENT®*** for any particular use, since its performance characteristics have not been validated for any specific organism. ***DECOCENT®*** 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 containing solution, decontaminates the samples by killing many microorganisms susceptible to sodium hydroxide while mycobacteria, that are resistant to alkaline pH, survive. Sodium hydroxide also decreases viscosity of the sputum and thus liquefies the samples. This facilitates the sedimentation of bacilli during centrifugation.

***Ingredients:***
One box of ***DECOCENT®*** DCC010contains:

- 75 units of 10mL decontamination solution in polypropylene tube, containing NaOH and phenol red as pH indicator.
- 74 units of10 mL, neutralization solution, in polypropylene tube, containing acid supplemented phosphate buffer.

Additional to the above materials DCC030 contains 75 units of 30mL and DCC050 contains 75 units of 50mL conical centrifuge tube.

***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.
***DECOCENT®*** 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 that 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 laminary flow cabin, 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 holders adapted to the shape of the sampling tubes.
- If spills of the contaminated material occur, disinfect with 2.5% hypo-chloride solution.
- If decontamination of neutralization solution contacts skin, eyes or mucosal surfaces, wash immediately and thoroughly with water and seek immediate medical help.
- 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.

# DECOCENT®

***Storage instructions:***
Store at room temperature, in a dry place, in its original box.

***Indications of instability or deterioration:***
***DECOCENT®*** kit should not be used if above indicated volumes are not present in each tube and if there is turbidity or sediments in the solutions.

***List of materials provided:***
List of materials for processing one sample:

- 10mL decontamination solution (NaOH with phenol red as pH indicator) in polypropylene tube.
- 10mL neutralization solution (acid-supplemented phosphate buffer) in polypropylene tube.

In DCC010 each cardboard box contains 75 sets of the materials listed above.Centrifuge tubes for putting the samples are also provided in ***DECOCENT®*** kits with catalog no DCC030 (30mL tubes) and DCC050 (50mL tubes).

***Instructions for use:***
**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 centrifuge tube. If the sample is collected already in the centrifuge tube it can be used directly by adding the decontamination fluid.
2- Add all of the decontamination fluid (which is red in color) to the sample tube. Close the cap securely.
3- Homogenize the sample by shaking with a vortex and let it stand at room temperature for 15 minutes.
4- Add all of the neutralization fluid (which is transparent) to the sample tube. Close the cap securely.
5- Mix the sample by shaking with a vortex. (Formation of orange to pinkish color will indicate that the pH is adjusted properly.)
6- Spin the tubes for 15 minutes at 3000xg(if available, use a refrigerated centrifuge with airtight buckets to minimize killing mycobacteria due to heat formation.)
7- Carefully discard the supernatant into a container with disinfectant solution. Leave approximately 2mL of fluid over the sediment.
8- Resuspend the sediment by vortexing; this suspension can now be used for culture, microscopy and molecular diagnostic methods.

**Urine samples:**

It is recommended to obtain early morning urine to increase the chance of recovery of mycobacteria.
1- Transfer the urine sample from the collection cup into the centrifuge tube. (The tube can be filled up to the 50mL line or the 30mL line if using 30mL tube.)
2- Spin the tubes for 15 minutes at 3000xg.(If available, use a refrigerated centrifuge with airtight buckets to minimize killing mycobacteria due to heat formation.)
3- Continue processing, by starting from step 2, described above for other types of samples.

***List of materials that are not provided:***

- Centrifuge tubes for DCC010
- (Refrigerated) centrifuge
- Vortex
- Automatic pipettors
- Sterile pipette tips
- Disinfectant solution

***Quality control:***
Positive control: Respiratory secretions spiked with mycobacteria.
Negative control: Respiratory secretions spiked with *Escherichia coli* and *Staphylcoccus 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 between 30 to 45 minutes. The procedure is performed at room temperature. It is recommended to spin in a centrifuge at 4°C to minimize killing of mycobacteria.

***Time restrictions:***
Decontamination time must be from 10 to 20 minutes. Shorter decontamination time may increase contamination rate. Extension of decontamination time may lower mycobacterial isolation rate.

***Limitations of the procedure:***
Some organisms other than mycobacteria may survive decontamination process.

***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.

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***Catalogue number:***
DCC010, DCC030, DCC050

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