AlphaTec™
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Directions For Use for the following:

AFB Base Decontamination Reagent

INTENDED USE
AFB Base Decontamination Reagent in combination with NALC is used in the qualitative procedure in the N-acetyl-L-cysteine (NALC) digestion and decontamination procedure of clinical specimens for the increased recovery of *Mycobacterium* spp.

SUMMARY
The decontamination and digestion procedure, utilizing the compound N-acetyl-L-cysteine (NALC) combined with sodium hydroxide and sodium citrate (trisodium citrate) solution, results in increased yields of tubercle bacilli. The NALC procedure utilizes N-acetyl-L-cysteine as a mucolytic compound by disrupting chemical bonds in mucus. The sodium hydroxide acts as a bacterial decontaminate and the sodium citrate solution stabilizes the NALC by chelating (binding) any heavy metal ions present in the specimen. Since the sodium hydroxide has a pH of approximately 13.00, it will kill bacteria (including mycobacteria after 15-20 minutes of exposure). As such, timing of the decontamination is critical to limit the amount of *Mycobacterium* spp. killed by the basic pH. Bringing the pH to a neutral range can stop the decontamination process. The NPC-67® Neutralizing Buffer or XPR-*PLUS*® Neutralizing Buffer can be used to neutralize the NALC reagents following the appropriate digestion and decontamination time, resulting in a pH below 8.10. Adding conventional M/15 Phosphate Buffer or phosphate buffered saline will result in a pH range of 9.40 to 12.20, requiring a titration to a neutral pH with 1N HCl or continued decontamination of *Mycobacterium* spp. will occur. Studies have documented that pH values above 8.10 are toxic to *Mycobacterium* spp., including *Mycobacterium tuberculosis*. Following the decanting step, PRB™ Pellet Resuspension Buffer is added to achieve a tight neutral pH value (6.80-7.10) in the specimen sediment, optimizing mycobacteria recovery.

FOR IN VITRO DIAGNOSTIC USE ONLY

PRECAUTIONS
The decontamination reagent contains a caustic chemical (sodium hydroxide).Use appropriate care in the handling of this reagent. All clinical specimens submitted for the diagnosis of tuberculosis and other *Mycobacterium* spp. must be treated with appropriate care so as not to contaminate other specimens or laboratory personnel. Use all approved and regulated equipment for processing and detection procedures.

STABILITY AND STORAGE
AFB Base Decontamination Reagent is stable to the stated expiration date when stored at the required temperature. Prior to opening, store at room temperature (15-30ºC). After opening, store between 2-8ºC. Do not freeze or heat above 30°C. Allow the product to come to room temperature prior to use.

USER QUALITY CONTROL
Any product showing cloudiness, turbidity, precipitation or coloration should be discarded. Quality controlled microorganisms should be utilized to verify procedures, media and reagents as appropriate for your laboratory's applicable regulatory agency or local procedural guidelines.

SPECIMEN COLLECTION AND PREPARATION
Appropriate specimens for the detection of *Mycobacterium* spp. should be collected according to prescribed standards and delivered to the laboratory in a safe and timely manner. Refer to local procedural guidelines for this information.

PROCEDURE
Materials Provided: AFB Base Decontamination Reagent.
Materials Not Provided: NALC ampules or tablets, centrifuge, vortex mixer, sterile pipettes, centrifuge tubes, neutralizing buffer, resuspension buffer, 1N HCl.

SPECIMEN PROCESSING
1. Line up specimens (in centrifuge tubes) in a biosafety hood.
2. Loosen specimen container caps. Work in sets equivalent to a centrifuge load.
3. Open the bottle labeled "AFB Base Decontamination Reagent" and either add 250 mg of NALC per 50 ml of AFB Base Decontamination Reagent, or place one tablet in each centrifuge tube containing a specimen. **Once dissolved, the AFB Base Decontamination Reagent / NALC solution will be stable for 72 hours. Store any unused portion at 2-8°C for up to 72 hours. Allow the refrigerated portion to come to room temperature prior to use.**
4. To the sterile 50 ml centrifuge tube containing the specimen to be digested, add an equal volume of the AFB Base Decontamination Reagent / NALC solution (up to 8 ml of specimen). If the specimen exceeds 8 ml, add a volume of AFB Base Decontamination Reagent / NALC equal to the volume of the patient sample, but spit it into two centrifuge tubes immediately prior to the addition of the neutralization buffer. Recombine the sediments after centrifugation and decantation.
5. Tighten the caps on the centrifuge tubes. Mix each specimen on a vortex until liquefied (30 seconds per specimen).
6. Allow each specimen to stand for 15-20 minutes. Vortex every 5 minutes during this step.
7. To complete the AFB diagnostic process, follow the neutralization and diagnostic procedures of your choice. Alpha-Tec Systems strongly recommends the use of either XPR-*PLUS* Neutralizing Buffer or NPC-67 Neutralizing Buffer along with PRB™ Pellet Resuspension Buffer.
   NOTE: Using M/15 Phosphate Buffer will result in a pH range that exceeds the tolerance of *Mycobacterium* spp. and will cause mycobacteria to die off. If M/15 Phosphate Buffer is used, titrate with 1N HCl and an appropriate pH indicator to ensure neutralization. Refer to the manufacturer's Directions For Use for the selected neutralization buffer's appropriate protocol.

PROCEDURE NOTES
1. Specimens contaminated with *Pseudomonas* spp. will need additional treatment with 5% Oxalic Acid (#0003447) or OxA® Oxalic Acid Reagent Kit (#0004805). Refer to the Oxalic Acid Directions For Use for complete instructions, or call Alpha-Tec Systems, Inc. Technical Services for information on the pH effects of the Oxalic Acid procedure and the appropriate buffering requirements.
2. The use of 0.2% Bovine Albumin Fraction V, (BA) is optional. However, if BA is not used, some type of material or CELL-BOND® Slides must be used to adhere the specimen pellet material to a microscope slide. Using a sterile pipette, add 1 or 2 drops of BA to the pellet. Shake gently by hand to mix.

EXPECTED RESULTS
To avoid the loss of any mycobacteria due to extended exposure to an elevated pH, specimens must be neutralized immediately following the addition of M/15 Phosphate Buffer. This neutralization can best be accomplished by titration with 1N HCl until the pH of the specimen falls below 8.10. A pH indicator can be added to the solution, or NAC-PAC® *RED* can be used in place of AFB Base Decontamination Reagent as it contains an integral pH indicator that visually confirms neutralization. Other buffers can be used to avoid the use of 1N HCl. Contact Alpha-Tec Technical Services for additional information.

LIMITATIONS OF PROCEDURES
This procedure is designed to be most effective with NPC-67 Neutralizing Buffer or XPR-*PLUS* Neutralizing Buffer. If M/15 Phosphate Buffer is used without proper subsequent neutralization, additional mycobacteria can be lost due to extended exposure to pH values above 8.10. To ensure this neutralization occurs, the pH must be immediately measured immediately following the addition of M/15 Phosphate Buffer and during titration.

SPECIFIC PERFORMANCE CHARACTERISTICS
AFB Base Decontamination Reagent was tested on clinical samples and recovered all culture appropriate *Mycobacterium* spp. when the designated procedures were followed.

BIBLIOGRAPHY
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8. Yegian, D., Budd V. 1952. "Toxic Effect of Sodium Hydroxide on Tubercle Bacilli." Am.J. Clinical Pathology. 22:456-460.

CONTACT
Alpha-Tec Systems, Inc. offers a complete line of reagents, stains, and QC1™ Quality Control Slides for AFB, Parasitology, Bacteriology, and Mycology processing, as well as O&P collection systems and concentration devices for Parasitology. For Technical Assistance, email Technical@AlphaTecSystems.com, and for Customer Service, email Sales@AlphaTecSystems.com, or call either [+1] 800.221.6058 (USA) or [+1] 360.260.2779 between 8AM and 4PM Monday through Friday, Pacific Time.

WARRANTY
This product is warranted by Alpha-Tec Systems, Inc. to perform as described in the labeling and literature supplied. Alpha-Tec Systems, Inc. disclaims any implied warranty or merchantability or fitness for any other purpose, and in no event shall Alpha-Tec Systems, Inc. be liable for any consequential damages arising out of aforesaid express warranty.

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PRODUCT CODES:
0003455 AFB Base Decontamination Reagent (3.0%), 10 x 500 ml
0003464 AFB Base Decontamination Reagent (2.0%), 18 x 100 ml

Update Glossary of Symbols with glossary that contains
Single use symbol

Manufactured by Alpha-Tec Systems, Inc.
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Vancouver, WA 98683 USA

GLOSSARY OF SYMBOLS

Batch code / Numéro de lot / Número de Lote / Numero di lotto / Lot Nummer / Lotnummer / Lotnummer / Šaržna številka / Número de lote

Catalog number / Référence du catalogue / Número de catálogo / Numero di catalogo / Katalognummer / Catalog nummer / Het aantal van de catalogus / Kataloška številka / Número de catálogo

In vitro diagnostic medical device / Pour usage diagnostique in vitro / Para uso diagnóstico in vitro solamente / Solo per uso diagnostico in vitro / Nur zur Verwendung als in vitro-Diagnostikum / Alleen voor in vitro diagnostisch gebruik / För invitrodiagnostik enbart / Samo za invitro diagnostiko / Apenas para uso em diagnóstico in vitro

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