LYE-NAC TB Reagents

Reagents for processing TB specimens

B2050 LYE-NAC BASE DIGESTANT 2.0% (5 X 50 ml)
B2250 LYE-NAC BASE DIGESTANT 2.0% (5 X 250 ml)
B2500 LYE-NAC BASE DIGESTANT (500 ml bottle) each

B2502 NALC Powder 250 mg x 25 vials (for 50 ml base digestant)
B2503 NALC Powder 1.25 g x 25 vials (for 250 ml base digestant)
B2504 NALC Powder 2.5 g x 16 tubes (for 500 ml base digestant)
B2501 NAC-EXTRA (for extra-viscous specimens) 10 x 150 mg vials

B4005 PHOSPHATE BUFFER 50 ml x 5
B4050 PHOSPHATE BUFFER 50 ml (25 bottles)
B4250 PHOSPHATE BUFFER 250 ml x 5
B4500 PHOSPHATE BUFFER 500 ML (each)

B3505 BOVINE SERUM ALBUMIN FRACTION V 3ml x 5
B3516 BOVINE SERUM ALBUMIN FRACTION V 3ml x 16

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INTENDED USE:

The LYE-NAC Reagents by Biorex Labs, are intended for the decontamination of Clinical specimens for laboratory mycobacterial culture. Clinical specimens suspected of containing mycobacteria, particularly sputum, are often contaminated by large numbers of bacterial flora.

Explanation, Summary and Principle

The N acetyl L cystein - sodium hydroxide (NAC-NaOH) method is one of the most satisfactory and widely employed digestion-decontamination procedures for the isolation of mycobacteria from clinical specimens. The limited exposure to sodium hydroxide is significantly lethal to the contaminating organisms, and less harmful to any mycobacteria that may be present in the clinical Specimen. In addition, N-acetyl L cysteine (NAC) is mixed with NaOH as a mucolytic agent to facilitate the recovery of trapped mycobacteria. NAC is added on the day of use, as its activity decreases if stored. It acts to liquefy the specimen by disrupting the disulfide linkages of the mucin aggregates. Trisodium citrate prevents NAC deactivation by any heavy metal ions when present. The method uses phosphate buffer pH (6.8-7.0) to neutralize the alkaline conditions and increases the sedimentation of mycobacteria by lowering the specific gravity of the solution. Finally, the sediment is diluted in 0.2% Bovine Serum Albumin which neutralizes the fatty acid toxicity, has a buffering action and thereby improves mycobacterial growth. Also, it allows for better adherence of the sediment to the medium and the glass slides for staining the sediment material.

Formulations (approximate)

Sterilized LYE-NAC Digestant :
NaOH ……..20.0 g
Trisodium Citrate…….. 14.7 g
Distilled Water to…….. 1.0 L
NAC-(N-Acetyl-L-Cysteine) 250 mg
(0.5 % in the final solution)
Sterilized Phosphate Buffer (pH 6.8-7.0)
Disodium Phosphate…….. 9.47gm
Monopotassium Phosphate... 9.07gm
Distilled water to…….. 1.0 L
Filter Sterilized Bovine Serum Albumin (BSA) 0.2%:
BSA (Fraction V)…….. 0.20gm
Sodium Chloride……...0.85 gm
Distilled Water…….. 100 ml

STORAGE AND SHELF LIFE:

Do not use the reagents if cloudy, discolored or a precipitate is evident. Solutions should not be used beyond their expiration date. Store the NAOH with NAC and Phosphate Buffer at 15-25°C Bovine Serum Albumin has an expiration date of 9 months from the date of manufacture and should be stored at 2-8°C.

Warnings and Precautions.

These reagents are for *in vitro* diagnostic use only. All clinical specimens are potentially infectious and should be handled in a biosafety hood**.** These products are to be used only by properly trained laboratory personnel only. Wear appropriate safety attire which includes goggles, mask, gloves and a lab coat. Follow CDC or other local and State regulations for handling and disposal of infectious waste. Sterilize all biohazard waste before disposal. NaOH exposure, longer than the protocol, is lethal to mycobacteria as well and should be avoided.

Materials required but not provided.

Centrifuge tubes waste disposal supplies and equipment such as mixers, biological safety hoods, glass slides, loops, diposable sterile pipettes, incinerators and incubators, etc. are not provided.

Procedure:

Collect the clinical specimen in a designated sterile container according to your laboratory protocol and should be processed as soon as possible. If the delay is inevitable, specimen should be refrigerated. Prepare enough fresh digestant solution for a single day use only.

1. Prepare the activated NaOH-NAC solution by dissolving the contents of one NAC Vial (included) to a single bottle of NaOH-Base Digestant. The quantity of NAC is premeasured to yield a final working solution of 0.5%.
2. Transfer 5-10ml of sputum specimen and an equal amount of the activated digestant into a 50 ml, aerosol-free, screw-capped centrifuge tube.
3. Invert the tube a few times. Vortex the tube for 20 seconds to liquefy the specimen. For very viscous specimen, additional 150 mg of NAC (No.B2501) may be added.
4. Allow the tube to sit in a rack at room temperature for 15 minutes.
5. Fill the tube with Phosphate Buffer (B4050 or B4500) to the 50 ml mark. Swirl to mix well. To avoid cross-contamination, do not touch the lip of the specimen container with the reagent bottles.
6. Centrifuge for 15 minutes at ≥ 3000 X *g*.
7. Aseptically decant the supernatant, without dislodging the sediment, into a splash proof waste container containing Alkyl dimethyl benzyl ammonium saccharinate (Amphyl). Wipe the lip of the container with disinfectant. Do not allow the disinfectant to enter the tube.
8. Re-suspend the sediment in 1-1.5 ml of sterile 0.2% Bovine Serum Albumin (B3503). Mix gently to dislodge the sediment.
9. Use the suspension to inoculate the appropriate medium for culture or susceptibility testing according to your protocols for mycobacterial culture.
10. Make smears for AFB or fluorescence staining.

Limitations:

Digestant treatment longer than the prescribed time is lethal to mycobacteria and may diminish the recovery significantly. Do not Use the activated reagents beyond the day of mixing. NAC, once mixed with NaOH, becomes ineffective after 18-24 hrs.

Quality Control:

The product is tested only for its ability to decontaminate usual bacterial flora.
1. Prepare a 3ml suspension each of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in saline. Adjust the turbidity to match that of a 0.5 McFarland standard.
2. Add 3ml of NaOH-NAC Digestant to each of the suspension and incubate atroom temperature for 15 minutes.
3. Neutralize the suspension by adding 20 ml of Phosphate Buffer and mix / vortex.
4. Spin for 10 minutes ≥ 3000 X *g*.
5. Decant the supernatant and resuspend the sediment in 0.5 ml of 0.2% albumin.
6. Plate 10 µl onto a blood agar plate and streak with a loop. Incubate 18 hrs. at 35°C. Both *P. aeruginosa* and *S. aureus* should be partially to completely inhibited after 24 hours of incubation.

Note: The alkaline pH is the inhibitory factor in NaOH-NAC digestant, therefore, some of the pH tolerant organism such as *Staph. aureus* ATCC 25923 may still survive. Up to 50 colonies for *S. aureus* is acceptable. For *Pseudomonas aeruginosa* up to 5 colonies is an acceptable level of decontamination.

REFERENCES:

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4. Isenberg, H.D. Clinical Microbiology Procedures Handbook, Vol. I & II. American Society for Microbiology, Washington, D.C.
5. Kubica, G. P., W. E. Dye, M. L. Cohn, and G. Middlebrook.1963. Sputum digestion and decontamination with N-acetyl-Lcysteine-sodium hydroxide for culture of mycobacteria. Am. Rev. Respir. Dis. 87:775-779.

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