K7R25050 NAOH-NALC RED DIGESTANT KIT 2.5% 5 X 50ML
K7R25200 NAOH-NALC RED DIGESTANT KIT 2.5% 5 X 200 ML
K7670050 PHOSPHATE BUFFER 50 ML (25 bottles)
K7670500 PHOSPHATE BUFFER 500 ML (each)
K7680003 BOVINE SERUM ALBUMIN FRACTION V 3ml

INTENDED USE:
Because mycobacteria generally grow much slower than other bacterial species and specimens containing mycobacteria also contain many other types of bacteria, recovery is often difficult. The use of n-acetyl-l-cystine is widely used for decontamination and liquefication of the specimens allowing greater yield of the slow growing mycobacteria . N-Acetyl-L-Cysteine (NALC), when combined with NaOH, facilitates decontamination by digesting mucopurulent specimens which allows the NaOH to penetrate. Sodium citrate, stabilizes the NALC, allowing it to work properly. The final combined solution is only usable for 24 hours after adding the NALC. For this reason, the NALC is packaged separately, to allow mixing only as needed. Phosphate Buffer lowers the specific gravity of the specimen and gently neutralizes the specimen after decontamination. The red indicator makes it easy to determine the correct pH. Bovine Serum Albumin is added to the sediment after centrifugation to enhance the growth of mycobacteria. Bovine Serum Albumin also assists in adhering the sediment material to the slide or solid media and increases the volume of material for culture.

APPROXIMATE FORMULATIONS:
NAOH-NALC DIGESTANT :
NaOH 2.5% (50%)
Sodium Citrate 2.94 % (50%)
Red dye (proprietary)
NALC- N-Acetyl-L-Cysteine to achieve a final dilution of 0.5 %
PHOSPHATE BUFFER:

Disodium Phosphate	9.47gm
Monopotassium Phosphate	9.07gm
Deionized Water	1000.0ml

BOVINE SERUM ALBUMIN:

Bovine Serum Albumin Fraction V	0.20gm
Sodium Chloride	0.85gm
Deionized Water	100.0ml

STORAGE AND SHELF LIFE:
Do not use any of the material if there are any signs of discoloration, contamination, deterioration or if the expiration has passed. The expiration date applies to the product when stored as directed. The NAOH-NALC kit and Bovine Albumin have an expiration date of 180 days from date of manufacture and should be stored at 2-8 degrees C. Store the NAOH-NAOH Kit with NALC and Phosphate Buffer at 15-30 degrees C.

CAUTIONS:
This product is for in vitro diagnostic use only and is to be used only by adequately trained and qualified laboratory personnel. All laboratory specimens should be considered infectious and handled according to approved laboratory guidelines for infectious material.
Sterilize all biohazard waste before disposal.

PROCEDURE:
Specimen should be collected in a sterile container. Transport specimens to the lab without delay. The specimen should be refrigerated if processing will be delayed. Work within a biological safety cabinet and wear gloves.
1. Prepare fresh digestant solution daily by dissolving one container (included) of NALC Reagent to one bottle of the NAOH-NALC Base Digestant. The correct size is included in the kit to make a final concentration of 0.5%. Prepare only what can be used in 24 hours.
2. Transfer 5-10ml of sputum specimen and an equal amount of the activated digestant into a 50ml, aerosol-free, screw-capped centrifuge tube.
3.. Vortex the centrifuge tube until the specimen is liquified. If specimen is extremely viscous, add a little more digestant.
4. Allow the tube to sit at room temperature (15-30 degrees C.) for 15-20 minutes, not longer.
5. Fill the tube with Phosphate Buffer until the red color disappears, indicating a neutral pH has been achieved. Swirl to mix. Avoid touching the lip of the specimen container with the reagent bottles.
6. Centrifuge at least 15 minutes at 3600Xg.
7. Asceptically decant the supernatant into a splash proof waste container containing a sterilant such as 5% phenol. Wipe the lip of the container with disinfectant. Do not allow the disinfectant to enter the tube.
8. Best: Resuspend the sediment in 1-2ml of 0.2% Bovine Serum Albumin. Swirl to mix.
Optional:If media will be inoculated immediately, the sediment may be suspended in sterile saline or water.
9. The suspension may then be used for smear prep, culture, or susceptability testing following protocol for mycobacterial cultures.
10. Follow CDC protocol for the interpretation of growth.

LIMITATIONS:
Timing is important during the digestion process. A digestion time of longer than 15 minutes should not be used. Many Mycobacterium spp. are killed by over decontamination. Occasional specimens are so contaminated with resistant bacteria that the decontamination process is not effective and the contaminating bacteria will overgrow the slower growing mycobacteria. Sediment material may be redigested and a selective medium, with antibiotics such as Lowenstein Jensen, Selective or Middlebrook 7H11, can be used to decrease the growth of contaminating organisms.

MATERIALS REQUIRED BUT NOT PROVIDED
Microbiological supplies and equipment such as vortex mixers, biological safety cabinets, centrifuge tubes, slides, media, loops, incinerators, incubators, pasteur pipets, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL:
NAOH-NALC Base Digestant is not a growth medium. The product is tested only for its ability to decontaminate.
1. Prepare a 3ml suspension of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Adjust the turbidity to match that of a 0.5 McFarland opacity standard.
2. Mix 3ml of TB Base Digestant to the suspension and incubate aerobically at 35 degrees C. for ten minutes.
3. Neutralize the suspension by adding 6ml of Phosphate Buffer and mix/vortex.
4. Spin for 10 minutes @ 2000 RPM.
5. Decant the supernatant and resuspend the sediment in 0.5 ml of 0.2% albumin.
6. Plate 10 ml onto a blood agar plate
4. Incubate aerobically at 35 degrees C. Both *P. aeruginosa* and *S. aureus* should be partially to completely inhibited after 24 hours of incubation.
Note: The elevated pH is the inhibitory factor in NaOH-NALC digestant, therefore, the pH tolerant organisms may still survive, particularly *Staph. aureus* ATCC® 25923, especially during the QC procedure when the process sample is plated on non-selective media. Up to 50 colonies for 2 and 2.5%, and 5 colonies for 5% reagents is acceptable. For *Pseudomonas aeruginosa* , complete inhibition is expected for 4% reagent. For 2 and 2.5% reagents, up to 5 colonies is acceptable.

REFERENCES:
1. Murray, P.R., et al. 1995. Manual of Clinical Microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. Forbes, B.A., et al. 1990. Bailey and Scott's Diagnostic Microbiology, 8th ed. C.V. Mosby Company, St. Louis, MO.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook, Vol. I & II. American Society for Microbiology, Washington, D.C.

KEY SCIENTIFIC PRODUCTS
1113 EAST REYNOLDS ST.
STAMFORD, TEXAS 79553
WWW.KEYSCIENTIFIC.COM
Voice 800-843-1539
Fax 888-440-4208