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A Geno Technology, Inc. (USA) brand name

Sputo-LR™

A Liquefaction Reagent for Sputum

(Cat. # 786-1086, 786-1087)



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INTRODUCTION

Sputo-LR™ is a sputum liquefying reagent for sputum and other mucoid fluids that is useful for the isolation of pathogenic or saprophytic bacteria, fungi, yeast, and epithelial cells. Sputo-LR™ is a concentrated sterile solution of Cleland's Reagent (dithiothreitol, DTT) in phosphate buffer at pH 7.0 and is for processing samples from sputum for smears and culture.

Sputum or phlegm is the discharge that is expectorated from the respiratory system. It is a heterogeneous and viscous substance that differs in appearance (mucoid, purulent, mucopurulent and blood-streaked). Consequently, the various host components and organisms in the sputum are not evenly distributed which makes it difficult to process multiple, equivalent samples for research and cryopreservation.

Sputo-LR™ chemically digests sputum to decrease viscosity and create a homogenous suspension that allows each sample to be an equivalent representation of the original sputum specimen. This chemical digestion or sputum liquefaction is accomplished with DTT. The active component of DTT is a sulphhydryl group, which reduces the mucoprotein cross-linking disulfide bonds. A small amount of DTT is added to the specimen, so it does not affect morphology, growth or fluorescent antibody staining of pathogens in sputum.

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-1086	Sputo-LR™	10ml
786-1087	Sputo-LR™	20 x 10ml

STORAGE CONDITIONS

Protect from light and store in refrigerator (4°C). Unopened vials are stable for 12 months from date of manufacture. Once a vial is opened the unused concentrate should be purged with an inert gas (e.g. N₂). Diluted solutions, stored aseptically under an inert gas, are stable for up to 48 hrs at 4°C.

PROTOCOLS

Preparation of Sputo-LR™

Aseptically dilute the contents of one vial (10ml) to 100ml with sterile distilled water. Swirl gently to dissolve any crystals. Appearance should be clear and colorless; filter if cloudy.

NOTE: *The diluted Sputo-LR™ should be used immediately or can be stored at 4°C under N₂ for 48 hrs.*

Sputum Liquefaction Protocol

A. To prepare sputum samples:

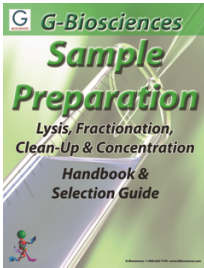
1. The sputum sample is expectorated into a sterile screw-cap centrifuge tube
2. Sputum samples can be washed with sterile saline to remove adherent saliva. Add approximately 5 times the volume of 0.85% sterile saline and agitate the sample. Drain off saline with a sterile Pasteur pipette.
3. Overlay the sputum sample with an equal volume of diluted Sputo-LR™.
NOTE: *Allow sputum specimen and Sputo-LR™ to come to room temperature for effective digestion.*
4. Shake well or vortex for 30 seconds.
5. Incubate at room temperature for 15 minutes.
6. Centrifuge the mixture for 5 min at 1500 rpm to pellet bacterial cells and discard the supernatant.
7. For the isolation of predominant organisms: re-suspend the pellet in a small amount of Sputo-LR™. The volume of diluents used will depend on the pellet volume and the final concentration desired.
8. A dilution of 1:100 with inoculums of 0.01 ml is recommended for colony counting. For an accurate count serial dilutions should be made.

B. To prepare cultures for acid-fast bacilli:

1. Liquefy the sputum sample and centrifuge as above (steps 1-6).
2. Decontaminate the specimen by re-suspending the pellet in 5-10 ml of 1% NaOH.
3. Vortex for 1 min.
4. Centrifuge the mixture for 15 min at 3000 rpm and discard the supernatant.
5. Rinse the pellet twice in 10 ml of sterile saline or diluted Sputo-LR™ to remove residual NaOH.
6. Re-suspend the pellet in 0.5 ml of diluted Sputo-LR™.
7. Culture for acid-fast bacteria on appropriate media.

RELATED PRODUCTS

Download our Sample Preparation Handbook



<https://info2.gbiosciences.com/complete-sample-preparation-handbook>

For other related products, visit our website at www.GBiosciences.com or contact us.

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