GeneReleaser® Protocol for Bronchoalveolar Lavage and Sputum

GeneReleaser® is composed of proprietary polymeric materials that quickly facilitate the release of nucleic acids from cells in a form suitable for PCR. By segregating inhibitors that are released during lysis as well as preservation agents that may interfere with amplification, GeneReleaser® provides amplifiable nucleic acids from minute amounts of material. The protocols for Bronchoalveolar Lavage and Sputum samples are developed by GeneReleaser® users and have not been validated by BioVentures.

GeneReleaser® Protocol for Bronchoalveolar Lavage Specimens

1. Centrifuge BAL specimens at 1,500 x *g* for 5 min.
2. Thoroughly resuspend the contents of the GeneReleaser® tube by inverting 10-20 times or vortexing briefly.
3. Add 20ul of GeneReleaser® to 10ul of the sediment in an amplification tube.
4. Place samples onto thermal cycler, with a heated lid, with the following GeneReleaser® program:

Step	Temperature	Time
1.	65ºC	30 sec.
2.	8ºC	30 sec.
3.	65ºC	90 sec.
4.	97ºC	180 sec.
5.	8ºC	60 sec.
6.	65ºC	180 sec.
7.	97ºC	60 sec.
8.	65ºC	60 sec.
9.	80ºC	hold

5. Once program is completed, sample is ready to use as PCR template.
6. Add appropriate volume of mastermix (of 80µl of a 1.25X master mix containing all components for the amplification)
7. Perform amplification reaction according to your optimized protocol.

GeneReleaser® Protocol for Sputum

1. Solubilize sputum sample in 200ul aliquots with 100ul of 2% N-acetyl- cysteine for 20 minutes at 37ºC
2. Add 4% NaOH to the sample tube and incubate for 20 minutes at 37ºC.
3. Buffer with 200ul of 1M Tris-HCl, pH7.0.
4. Pellet bacterial cells by centrifugation at 15,800 x g for 30 min.
5. Thoroughly resuspend the contents of the GeneReleaser® tube by inverting 10-20 times or vortexing briefly.
6. Add 20 ul of GeneReleaser to 5ul of cell pellet in an amplification tube.
7. Place samples onto thermal cycler, with a heated lid, with the following GeneReleaser® program:

Step	Temperature	Time
1.	65ºC	30 sec.
2.	8ºC	30 sec.
3.	65ºC	90 sec.
4.	97ºC	180 sec.
5.	8ºC	60 sec.
6.	65ºC	180 sec.
7.	97ºC	60 sec.
8.	65ºC	60 sec.
9.	80ºC	hold

8. Once program is completed, sample is ready to use as PCR template.
9. Add appropriate volume of mastermix (of 80µl of a 1.25X master mix containing all components for the amplification)
10. Perform amplification reaction according to your optimized protocol.

Microwave Lysis Protocol:
We have found that the microwave treatment of specimens affords a rapid sample preparation and facilitates the amplification of the more intractable types of specimens. The microwave protocol has not been validated for Bronchoalveolar Lavage or sputum samples.

A. Evaluation of microwave
Perform the following experiment to determine the optimal conditions for your tubes and microwave.

1. Place 40µl DI water in the same type of tube that you will be using for GeneReleaser® treatment.
2. Overlay each tube with mineral oil to prevent evaporation.
3. Close the tubes, place in microwave safe rack (polyethylene or propylene) and heat on high for 5 minutes.
4. If any caps pop or tubes distort in any manner, then place a separate beaker in the microwave with 150ml of room temperature DI water and repeat the above 3 steps, the beaker of water serves as a heat ballast.
5. If tubes open or distort, reduce the power by 10% increments and increase time by 1-minute increments repeating step 4 until tubes no longer open or distort.

Note: Make sure the racks used in this procedure are MICROWAVE SAFE! The 0.2ml tube racks provided by Perkin Elmer for use with their 9600 and 2400 instruments are NOT microwave compatible, they will melt.

B. Microwave Protocol
1. Perform microwave procedure above for time and power conditions
2. Place 1µl of specimen with 20µl of GeneReleaser® into either a 0.5ml PCR tube or 1.5ml tube.
3. Vortex the tubes containing specimen and GeneReleaser® for ~10 seconds.
4. Overlay with mineral oil to prevent samples from evaporating.
5. Place the closed tubes in a microwave safe polyethylene or propylene rack. Make sure that the lids are loosely closed. If lids are closed too tightly tubes could rupture.
6. Place the rack in a microwave oven and heat at maximum power setting (setting should be based on the microwave evaluation results) for 5-7 minutes. Typically, 5 minutes if wattage is 900 or higher and 7 minutes if wattage is 500.
7. Remove rack from microwave and centrifuge the tubes at 5000xg for 5 minutes. After centrifuging samples, remove supernatant and use as DNA template.
8. Perform the amplification reaction.

References:

Amicosante M, Richeldi L, Trenti G, Paone G, Campa M, Bisetti A, Saltini C. Inactivation of polymerase inhibitors for Mycobacterium tuberculosis DNA amplification in sputum by using capture resin. J Clin Microbiol. 1995 Mar;33(3):629-30.

Rabodonirina M, Raffenot D, Cotte L, Boibieux A, Mayençon M, Bayle G, Persat F, Rabatel F, Trepo C, Peyramond D, Piens MA. Rapid detection of Pneumocystis carinii in bronchoalveolar lavage specimens from human immunodeficiency virus-infected patients: use of a simple DNA extraction procedure and nested PCR. Journal of Clinical Microbiology. 1997 Nov;35(11):2748-51.