GenePurgeDirect® DNA/RNA Releasing Agent

Mycobacterium Tuberculosis

Version: 1.0
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Description

GenePurgeDirect® 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, GenePurgeDirect® provides amplifiable nucleic acids from minute amounts of material. The protocols for MTB have been developed by GenePurgeDirect® users and have not been validated by NimaGen.

Protocols

GenePurgeDirect® Protocol for NaOH Decontaminated MTB material

1. Concentrate or enrich MTB by standard method (ASM or other) from sputum, urine, etc.
2. Transfer suitable aliquot to a standard amplification tube.
3. Centrifuge for 1 minute at max speed to pellet the sample, discard the supernatant.
4. If NaOH decontamination was employed, neutralize excess NaOH by three washes of 20 volumes (20 x the estimated volume of the pellet obtained from enrichment) of 1M tris buffer pH 8.3. Centrifuge after each wash to re-pellet the MTB containing sample.
5. Completely remove the supernatant from the last wash.
6. Thoroughly resuspend the contents of the GenePurgeDirect® tube by inverting 10-20 times or vortexing briefly.
7. Add 20µl of GenePurgeDirect® to cell pellet (usually around 10µl pellet volume) and vortex vigorously to resuspend the cell pellet.
8. Place samples onto thermal cycler, with a heated lid, with the following GenePurgeDirect® 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

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

* GenePurgeDirect® treatment can also be performed in the microwave, see procedure on page 2.

GenePurgeDirect® Protocol for Nont decontaminated MTB material

1. Transfer suitable aliquot of enriched MTB sample prior to decontamination step to a standard amplification tube.
2. Resuspend the GenePurgeDirect® mixture by vortexing 2-3 seconds or inverting 5-10 times.
3. Add 20µl of GenePurgeDirect® suspension to the sample and vortex vigorously to resuspend the cell pellet.
4. Place samples onto thermal cycler, with a heated lid, with the following GenePurgeDirect® 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 (80µl of a 1.25X master mix containing all components for the amplification)
7. Perform amplification reaction according to your optimized protocol.

* GenePurgeDirect® treatment can also be performed in the microwave, see procedure on page 2.

8. Serial dilutions or multiple volumes of the MTB enrichments may be required to establish optimal preparation.

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.

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 GenePurgeDirect® 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!

B. Microwave Protocol

1. Perform microwave procedure above for time and power conditions
2. Place 1µl of specimen with 20µl of GenePurgeDirect® into either a 0.5ml PCR tube or 1.5ml tube.
3. Vortex the tubes containing specimen and GenePurgeDirect® 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 resuls) 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:

Linton CJ, Jalal H, Leeming JP, Millar MR. Rapid discrimination of Mycobacterium tuberculosis strains by random amplified polymorphic DNA analysis. Journal of Clinical Microbiology 1994 Sep;32(9):2169-74.

Yamamoto G, Shirakawa T, Nishiyama K. Studies on the Elimination of PCR-Inhibitors in Clinical Materials and Direct Detection of Mycobacterium Tuberculosis DNA by an Anion Binding Resin. Kobe Daigaku Igakubu Hoken Gakka Kiyo 1998 (14): 125-129.