GenePurgeDirect® DNA/RNA Releasing Agent

Hepatitis

Version: 1.0
Revision date: 31-07-2014

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 Hepatitis have been developed by GenePurgeDirect® users and have not been validated by NimaGen.

Protocols

GenePurgeDirect® Protocol for DNA from Hepatitis virus

DNA from Hepatitis can be obtained from a number of sample types; this protocol addresses the amplification of the virus from serum or plasma. If serum aggregation occurs (especially with microwave protocol), we suggest the addition of fetal calf serum at a ratio of 1:1 with your serum.

1. Obtain plasma or serum by standard collection techniques.
2. Transfer 5µl of plasma/serum to a standard amplification tube.
3. Thoroughly resuspend the contents of the GenePurgeDirect® tube by inverting 10-20 times or vortexing briefly.
7. Add 15µl of GenePurgeDirect® to the plasma/serum sample and vortex briefly for 1-2 seconds.
8. Treat samples using either the thermal cycler program (below) or the microwave protocol (page2)
9. 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 noting the following:
    - The first cycle should have a denaturing time of 2-4 minutes at 94-95ºC.
    - The annealing temperature should be no higher than 55ºC.
    - For some primer sets a re-titration of the magnesium concentration may be required.
    - Appropriate controls treated with GenePurgeDirect® should also be analyzed.
    - For very low copy of the virus 40-50 cycles of amplification may be required.

GenePurgeDirect® Protocol for RNA from Hepatitis Virus

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10. Following the thermal cycler or microwave treatment, add an additional 20µl of RNase free water to the treated sample tubes.
11. Centrifuge at 12,000 x g for 5 minutes.
12. Transfer 10-20µl of the supernatant to a new amplification tube.
13. Perform reverse transcriptase reaction according to the manufacturer's protocol.
14. Using the cDNA obtained, amplify the specimens according to your optimized protocol noting the following:
    - The first cycle should have a denaturing time of 2-4 minutes at 94-95ºC.
    - The annealing temperature should be no higher than 60ºC.
    - For some primer sets a re-titration of the magnesium concentration may be required.
    - Appropriate controls treated with GenePurgeDirect® should also be analyzed.
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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 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:
Kramvis A, Bukofzer S, Kew MC. Comparison of hepatitis B virus DNA extractions from serum by the QIAamp blood kit, GenePurgeDirect, and the phenolo chloroform method. Journal of Clinical Microbiology. 1996 Nov;34(11):2731o 3.