

GeneReleaser®

DNA/RNA Releasing Agent

GeneReleaser® Protocol for Hepatitis Virus

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 Hepatitis have been developed by GeneReleaser® users and have not been validated by BioVentures.

GeneReleaser® 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 GeneReleaser® tube by inverting 10-20 times or vortexing briefly.
7. Add 15µl of GeneReleaser® 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 GeneReleaser® program:

Step	Temperature	Time
1.	65°C	30 sec.
2.	8°C	30 sec.
3.	65°C	90 sec.
4.	97°C	180 sec.
5.	8°C	60 sec.
6.	65°C	180 sec.
7.	97°C	60 sec.
8.	65°C	60 sec.
9.	80°C	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 94-95°C.
 - The annealing temperature should be no higher than 55°C.
 - For some primer sets a re-titration of the magnesium concentration may be required.
 - Appropriate controls treated with GeneReleaser® should also be analyzed.
 - For very low copy of the virus 40-50 cycles of amplification may be required.

GeneReleaser® Protocol for RNA from Hepatitis Virus

RNA 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 GeneReleaser® tube by inverting 10-20 times or vortexing briefly.
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5.	8°C	60 sec.
6.	65°C	180 sec.
7.	97°C	60 sec.
8.	65°C	60 sec.
9.	80°C	hold

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 94-95°C.
 - The annealing temperature should be no higher than 60°C.
 - For some primer sets a re-titration of the magnesium concentration may be required.
 - Appropriate controls treated with GeneReleaser® should also be analyzed.
 - For very low copy of the virus 40-50 cycles of amplification may be required.

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 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:

Kramvis A, Bukofzer S, Kew MC. Comparison of hepatitis B virus DNA extractions from serum by the QIAamp blood kit, GeneReleaser, and the phenol-chloroform method. *Journal of Clinical Microbiology*. 1996 Nov;34(11):2731-3.

If you have further questions, please contact us at support@bioventures.com. You may also contact us via fax (1-877-286-0330) or through our toll-free phone number (1-877-852-7846).