

GeneReleaser® Hepatitis C Protocol

RNA from Hepatitis C can be obtained from a number of different sample types using GeneReleaser®. This procedure addresses the amplification of the virus from serum or plasma.

1. Obtain plasma or serum by standard collection techniques
2. For maximum sensitivity, treat 5µl of serum with 15µl of resuspended GeneReleaser®.
3. Vortex the mixture briefly, i.e. 1-2 seconds
4. Overlay with mineral oil.
5. Treat the specimens using either the thermocycle lysis program or by the microwave protocol.
6. Following the lysis treatment, add an additional 20µl of RNase free water to the tubes and centrifuge at 12,000 x g for 5 minutes.
7. Transfer 10-20µl of the supernatant from step 6 to a fresh tube.
8. Perform reverse transcriptase reaction according to the source of your reverse transcriptase enzyme vendor's protocol
9. Using the cDNA obtained in step 8, 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°C.
 - The annealing temperature should be no higher than 60°C.
 - For some primer sets a retitration of the magnesium concentration may be required.
 - Appropriate controls treated with GeneReleaser® should also be analyzed.
 - For very low copies of the virus 40-50 cycles of amplification may be required.

Note: If serum aggregation occurs (esp. with microwave protocol), we suggest the addition of fetal calf serum at a ratio of 1:1 with your serum.

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).