

Technical Resources

GeneReleaser® General Protocol

GeneReleaser® is a proprietary reagent which releases DNA from whole blood, cell cultures, bacterial colonies and the like. Lysis is accomplished directly in the amplification tube on a thermocycler. Typically, 1µl of specimen is lysed in a total volume of 20µl and amplification reagents are subsequently added to initiate amplification. GeneReleaser® sequesters cell lysis products which might inhibit polymerases and improves amplification yield and specificity. GeneReleaser® greatly simplifies the amplification of genomic DNA by avoiding the requirement to purify DNA. This avoids lengthy protocols and excessive sample manipulations which may introduce contamination.

Materials and Equipment:

Kit components:

1. GeneReleaser® is provided as a ready to use stock. There are 4 X 2ml tubes sufficient for 400 assays. Store at 4°C. DO NOT FREEZE!
2. A thermocycler profile is provided for the use of GeneReleaser®.

Other Equipment:

3. A programmable thermocycler and accessory reagents and equipment for amplification.

General:

It is assumed that the user is familiar with the programming and use of thermocyclers and the reactions which can be performed on them. No license to use PCR is given or to be implied by this product or these instructions. A limited license for PCR can be obtained with the purchase of PCR reagents or thermocycling devices from Perkin Elmer.

Whole Blood Procedure:

1. Place 1µl of whole blood into the bottom of each thermocycling tube for each specimen.
2. Thoroughly resuspend the contents of the GeneReleaser® tube by inverting 10-20X.
3. Add 20µl of the resuspended GeneReleaser® to each tube.
4. Perform the thermocycler program described below.
5. Perform the amplification reaction according to your optimized protocol.

Bacterial Colony Procedure:

1. Place a single well isolated colony into the bottom of a thermocycler tube for each specimen.
2. Perform steps 2-5 as per these steps in the Whole Blood Procedure above.

Tissue Culture Procedure:

1. Place 1µl of cells at a density of 10^3 - 10^8 cells/ml into the bottom of a thermocycler tube for each specimen.
2. Perform steps 2-5 as per these steps in the Whole Blood Procedure above.

Thermocycler Program:

NOTE: Reaction volumes and concentrations should be adjusted proportionally to allow for the 20µl GeneReleaser® volume. In some instances a new magnesium titration may need to be performed to assure optimum amplification. We suggest the range of 1.5mM - 4.0 mM Mg in 0.5mM increments for this titration.

Following the thermocycler manufacturer's procedure and using the default transition rates between temperatures, enter and run the following program:

Note: When using the Perkin Elmer GeneAmp® PCR system 9600 or 2400 all times below should be reduced by 50%.

Overlay the specimens with mineral oil.

Step	Temperature	Heating Rate
1.	65°C	hold 30 sec.
2.	8°C	hold 30 sec.
3.	65°C	hold 90 sec.
4.	97°C	hold 180 sec.
5.	8°C	hold 60 sec.
6.	65°C	hold 180 sec.
7.	97°C	hold 60 sec.
8.	65°C	hold 60 sec.
9.	80°C	hold.
10.	Adjust temperature as necessary.	
11.	Add amplification reagents without vortexing or otherwise mixing the amplification tube contents.	
12.	Begin your optimized amplification. NOTE: It is very important that the very first denaturing step of the first cycle be at 95°C for 2-5 minutes (or follow Manufacturer recommendations for your hot start protocol.) depending on brand of cycler, reaction volumes etc.	

Performance Characteristics:

GeneReleaser® is designed for release of genomic DNA. If low copy number DNA is being processed i.e. virally infected cells, then cellular enrichment should be performed.

GeneReleaser® Tips

We have found that certain actions are essential for the proper performance of GeneReleaser®. These are described below:

- I. The sequence of reagent additions of the reaction components is critical. Additions should be performed in the following order:
 - A. Use **1µl** of whole blood or cells at a density of $\sim 10^8$ /ml.
 - B. Add 20µl of GeneReleaser® (for standard 100µl amplifications).
 - C. **DO NOT** vortex or mix components after steps A&B above!
 - D. Lyse the cells using the thermocycler program protocol.
 - E. Add amplification reagents (**DO NOT** vortex or mix).
 - F. Perform amplification. **NOTE: It is very important that the very first denaturing step of the first cycle be at 95°C for 2-5 minutes (or follow Manufacturer recommendations for your hot start protocol) depending on brand of cycler, reaction volumes, etc.**
- II. The specimen and GeneReleaser® volumes may be adjusted. However, no less than 5µl of GeneReleaser® or more than 5µl of specimen should ever be used.
- III. The volume of GeneReleaser® used to accomplish cell lysis should be compensated for by deducting an equivalent volume of H₂O from the components of the amplification reagents in order to maintain their appropriate concentrations in the final reaction volume.
- IV. Amplification reaction volumes may be reduced from the typical 100µl volume to as little as 25µl as long as proportionate reductions are made with respect to specimen and GeneReleaser® volumes.
- V. The original thermocycler program has been modified to an 80°C hold in order to obtain better amplification from denser tissue materials and to facilitate an initiation of the amplification cycles under conditions which minimize non-specific annealing of primers.
- VI. It must be emphasized that if, upon use of this product, the expected bands are not observed, then a magnesium titration should be performed. If this fails to produce the desired bands, then reduction of the annealing temperature by 5-10°C should be employed in conjunction with a magnesium titration. If either of these should fail, we will be glad to develop an optimized procedure for you.
- VII. If the GeneReleaser® treated specimens cannot be amplified after performing the procedure, specimens may be stored at either 4°C or -20°C until they can be amplified. Prior to amplification, stored specimens should be heated to 80°C and the amplification begun using steps 10-12 of the thermocycler program.

Microwave Procedure

Evaluation of Microwave

To evaluate your microwave, please perform the following experiment:

- A. Place 40µl of DI water in the same size and type of tube as for PCR.
- B. Overlay each tube with mineral oil.
- C. Close the tubes, place in microwave safe rack, and heat on HIGH for 5 minutes.
- D. If any caps pop or tubes distort in any manner, then place a separate beaker in the microwave with 150mls of ambient temperature DI water and repeat as above. The beaker of water serves as a heat ballast.
- E. If tubes open or distort after performing D. above, then reduce power by 10% increments and increase time by 1 minute increments repeating D. until tubes no longer open or distort.

Note: The 0.2ml tube racks provided by Perkin Elmer for use with their 9600 and 2400 instruments are NOT microwave compatible.

THEY WILL MELT!!

Microwave Protocol

1. We have found that microwave treatment of specimens affords a more rapid sample preparation and facilitates the amplification of the more intractable types of specimens.
2. Place 1µl of specimen with 20µl of GeneReleaser® into either a 0.5ml or 0.2ml PCR tube. **NOTE: It is extremely important not to use tubes any larger than these; as the samples will be boiled away or larger tubes will rupture!!**
3. Unlike the thermocycler program, vortex the tubes containing specimen and GeneReleaser® for ~10-30 seconds.
4. An oil overlay is optional.
5. Place closed tubes in polyethylene or polypropylene racks.
6. Place rack in microwave oven and heat at maximum power setting for 5-7 minutes. 5 minutes if wattage is 900 or higher, 7 minutes if wattage is 500. 4500 watt-minutes is the optimum.
7. Remove rack from microwave and place tubes on preheated thermocycler at 80-90°C.
8. Add PCR master mix and begin amplification cycles. **NOTE: It is very important that the very first denaturing step of the first cycle be at 95°C for 2-5 minutes (or follow Manufacturer recommendations for your hot start protocol.) depending on brand of cycler, reaction volumes, etc.**

If you ever have any questions regarding the use of GeneReleaser® or reaction conditions, please call or fax us. If you can spare either specimens (non-infectious) or primers, we will gladly assist in developing an optimized protocol.

BioVentures, Inc.
Phone: 1-800-235-8938
Revised 09/29/08

Fax: 1-615-896-4837

Revised 09/29/08