

GeneReleaser® Auxiliary 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 thermocycle profile is provided for the use of GeneReleaser®.
3. Other Equipment: A programmable thermocycler and accessory reagents and equipment for amplification.

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 thermocycle 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 thermocycle 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 10³-10⁸ cells/ml into the bottom of a thermocycle tube for each specimen.
2. Perform steps 2-5 as per these steps in the Whole Blood procedure above.

Thermocycle 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 94°C for 2-5 minutes 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.

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).