

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 thermocycle 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 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 10^3 - 10^8 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.