GeneReleaser®

DNA/RNA Releasing Agent

GeneReleaser® General Protocol

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 and preservation agents that may interfere with amplification, GeneReleaser® provides amplifiable nucleic acids from minute amounts of material.

GeneReleaser® General Protocol

1. Place 1-5µl of whole blood / 1 isolated colony / 1ul of cells at 10^3-10^8 cells/ml into the bottom of a standard amplification for each specimen.
2. Resuspend the contents of the GeneReleaser® tube by inverting 10-20 times or briefly vortexing.
3. Add 20µl of the resuspended GeneReleaser® to each tube, pulse vortex briefly to mix.
4. Perform the thermal cycler protocol (A) or microwave protocol (B).
5. Once program is completed, centrifuge sample tubes at 5,000 x g for 1 minute.
6. Transfer supernatant into a new tube for use as template for PCR. Use 1-10µl of supernatant per 20-100µl amplification reaction.
    - PCR can be performed directly in the GeneReleaser® treatment tube; add amplification reagents for a final volume of 100µl with all components at a final concentration of 1X.
7. Perform amplification reaction according to your optimized protocol.
    - The first cycle should have a denaturing time of 2-4 minutes at 94-95ºC.
    - For some primer sets a re-titration of the magnesium concentration may be required.

(A) Thermal Cycler Lysis Protocol

Place samples onto thermal cycler, with a heated lid, with the following GeneReleaser® program:

Step	Temperature	Time
1.	65ºC	30 sec.
2.	8ºC	30 sec.
3.	65ºC	90 sec.
4.	97ºC	180 sec.
5.	8ºC	60 sec.
6.	65ºC	180 sec.
7.	97ºC	60 sec.
8.	65ºC	60 sec.
9.	80ºC	hold

(B) 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.

I. 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.

II. Microwave Protocol

1. Perform microwave procedure above for time and power conditions
1. Place 1µl of specimen with 20µl of GeneReleaser® into either a 0.5ml PCR tube or 1.5ml tube.
2. Vortex the tubes containing specimen and GeneReleaser® for ~10 seconds.
3. Overlay with mineral oil to prevent samples from evaporating.
4. 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.
5. 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.
6. Remove rack from microwave and centrifuge the tubes at 5000xg for 5 minutes. After centrifuging samples, remove supernatant and use as DNA template.
7. Perform the amplification reaction

General Tips:

1. For best performance make sure GeneReleaser® is mixed thoroughly; it may need to be shaken between samples.
2. The specimen and GeneReleaser® volumes may be adjusted, however use no less than 5ul of GeneReleaser®.
3. GeneReleaser® can be UV treated for 30 minutes prior to use if required.
4. The volume of GeneReleaser® used to accomplish lysis should be compensated for by deducting an equivalent volume of water from the components of the amplification reagents to maintain their appropriate concentrations in the final reaction volume.
5. If upon use of this product, the expected bands are not observed, then a magnesium titration (1.5mM – 4.0mM) should be performed. If this fails to produce the desired bands, then a reduction in annealing temperature by 5ºC to 10ºC should be employed in conjunction with the magnesium titration.
6. If the GeneReleaser® treated specimens need to be stored prior to performing the amplification reaction, store at 4ºC or -20ºC until the treated specimens can be amplified. Prior to amplification, stored specimens should be heated to 80ºC and the amplification begun using an initial denaturing step of at least 2 minutes.

Store at 4ºC. **DO NOT FREEZE**.

References:

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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).