

GeneReleaser®

DNA/RNA Releasing Agent

GeneReleaser® Protocol for Fresh or Frozen Tissue and Paraffin Embedded Tissue

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 as well as any preservation agents that may interfere with amplification, GeneReleaser® provides amplifiable nucleic acids from minute amounts of material. The protocols for Tissue/FFPE tissue are compiled from user-developed methods, see the listed references for additional information.

I. GeneReleaser® Protocol for Fresh/Frozen Tissue

Reagents: Sterile DI water
1X TE
Proteinase K (optional)
GeneReleaser®

Part I: Tissue Homogenization

1. Cut a 1mm³ thick section from either fresh or frozen tissue that has been rinsed with sterile water to remove any surface contamination.
2. Place the section into the bottom of a 1.5ml tube.
3. Add 25µl of 1X TE to the tube containing the sectioned tissue.
4. Mince the section of tissue by pushing a pestle against the tissue and twisting the pestle to compress the tissue against the walls of the tube. Ten thrusts with the pestle should be sufficient.
* Optional: 10 units of Proteinase K can be added and the tissue digested for 1-3 hours at 55°C.
5. Place the tube containing the homogenate at 4°C if subsequent sections need to be homogenized.

Part II: GeneReleaser® Treatment

6. Flick the homogenate tube 3 times then transfer 1µl of the homogenate to a 0.5ml amplification tube that is PCR compatible with your thermal cycler.
7. Resuspend the GeneReleaser® mixture by vortexing 2-3 seconds or inverting 5-10 times.
8. Add 20µl of GeneReleaser® suspension to the 1µl of homogenate in the PCR tube and tightly close the tube lid. Pulse vortex briefly to mix.
9. Place samples onto thermal cycler, with a heated lid, with the following GeneReleaser® program:

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

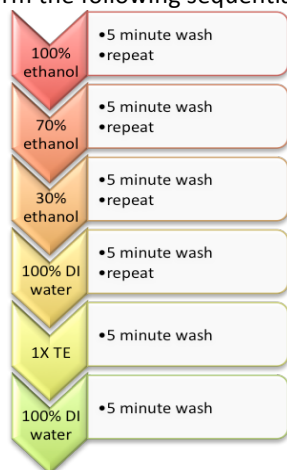
10. Once program is completed, centrifuge sample tubes at 5,000xg for 1 minute.
11. Transfer the supernatant to a new tube for use as the template for PCR. Use 1-10µl of supernatant per 20-100µl amplification reactions. *PCR can be performed directly in the GeneReleaser® treatment tube; add amplification reagents for a final volume of 100µl.
12. Perform amplification reaction according to your optimized protocol.

II. GeneReleaser® Protocol for Paraffin Embedded Tissue

Reagents: Sterile DI Water
Xylene (Acetone if lipid rich tissue)
Ethanol (100%, 70%, 30%)
1X TE
Proteinase K (optional)
GeneReleaser®

Part I: Deparaffinize tissue (either slide mounted or section form a block)

1. Perform 2 x 15 minute washes in xylene or equivalent by completely immersing the tissue section or slide in the wash agent. *Use 100% acetone wash 2 x 5 minutes for lipid rich tissue like brain tissue.
2. Perform the following sequential washes by completely immersing tissue section or slide in the wash agent:



Part II: GeneReleaser® Treatment

3. Resuspend the GeneReleaser® mixture by vortexing 2-3 seconds or inverting 5-10 times.
4. Combine tissue and 20ul of GeneReleaser® into a 1.5 ml tube. If tissue was slide mounted then use approximately 1mm² to 9mm² tissue scraping into the tube.
5. Using a pestle grind the section of tissue by pushing a pestle against the tissue and twisting the pestle to compress the tissue against the walls of the tube. Ten thrusts with the pestle should be sufficient.
*Optional: 10 units of Proteinase K can be added and the tissue digested for 30 minutes at 55°C.
6. Transfer as much of the tissue homogenate as possible to a new 0.5ml PCR tube.
7. Proceed with either the thermal cycler lysis protocol (as described on page 1) or the microwave lysis protocol (below).
8. After the lysis program is complete, add 25µl of 1XTE to the treated tissue.
9. Centrifuge sample tube at 5,000xg for 5 minutes.
10. Transfer the supernatant to a fresh tube for use as a template for PCR. Use 1-10µl of supernatant per 20-100µl amplification reactions.
11. Perform amplification reaction according to your optimized protocol.

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.

A. 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.

B. 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
-

References:

Annis SL, Dastoor FP, Ziel H, Daszak P, Longcore JE. A DNA-based assay identifies *Batrachochytrium dendrobatidis* in amphibians. *Journal of Wildlife Diseases*. 2004 Jul;40(3):420-8.

Flore O, Rafii S, Ely S, O'Leary JJ, Hyjek EM, Cesarman E. Transformation of primary human endothelial cells by Kaposi's sarcoma-associated herpesvirus. *Nature*. 1998 Aug;394(6693):588-92.

Nakata B, Wang YQ, Yashiro M, Nishioka N, Tanaka H, Ohira M, Ishikawa T, Nishino H, Hirakawa K. Prognostic value of microsatellite instability in resectable pancreatic cancer. *Clinical Cancer Research*. 2002 Aug;8(8):2536-40.

Toledo LF, Haddad CFB, Carnaval ACOQ, Britto FB. A Brazilian anuran (*Hylodes magalhaesi* : Leptodactylidae) infected by *Batrachochytrium dendrobatidis* : a conservation concern. *Amphibian and Reptile Conservation* 2006; 4(1):17-21.

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).