GenePurgeDirect® DNA/RNA Releasing Agent

FFPE and Tissue

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Description

GenePurgeDirect® 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 GenePurgeDirect® provides amplifiable nucleic acids from minute amounts of material. The protocols for Tissue/FFPE tissue are complied from user-developed methods see the listed references for additional information.

Protocols

I. GenePurgeDirect® Protocol for Fresh/Frozen Tissue

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

Part I: Tissue Homogenization

1. Cut a 1mm^3 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 1J3 hours at 55ºC.
5. Place the tube containing the homogenate at 4ºC if subsequent sections need to be homogenized.

Part II: GenePurgeDirect® 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 GenePurgeDirect® mixture by vortexing 2-3 seconds or inverting 5-10 times.
8. Add 20µl of GenePurgeDirect® 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 GenePurgeDirect® 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.
6.	80ºC	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 1J10µl of supernatant per 20J100µl amplification reactions. *PCR can be performed directly in the GenePurgeDirect® treatment tube; add amplification reagents for a final volume of 100µl.
12. Perform amplification reaction according to your optimized protocol.

II. GenePurgeDirect® Protocol for Paraffin Embedded Tissue

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

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

1. Perform 2 x 15 minute washed 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: GenePurgeDirect® Treatment

3. Resuspend the GenePurgeDirect® mixture by vortexing 2-3 seconds or inverting 5-10 times.
4. Combine tissue and 20ul of GenePurgeDirect® into a 1.5 ml tube. If tissue was slide mounted then use approximately 1mm^2 to 9mm^2 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 55ºC.

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 1J10µl of supernatant per 20J100µl 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 GenePurgeDirect® 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 1Jminute increments repeating step 4 until tubes no longer open or distort.

Note:!Make!sure!the!racks!used!in!this!procedure!are!MICROWAVE!SAFE!!

B.!!Microwave!Protocol!

1. Perform microwave procedure above for time and power conditions

1. Place 1µl of specimen with 20µl of GenePurgeDirect® into either a 0.5ml PCR tube or 1.5ml tube.
2. Vortex the tubes containing specimen and GenePurgeDirect® 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 looselyclosed. 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 5J7 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:!

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