GenePurgeDirect® DNA/RNA Releasing Agent

Mouse Tail

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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 and preservation agents that may interfere with amplification, GenePurgeDirect® provides amplifiable nucleic acids from minute amounts of material. This manual offers 2 alternate protocols, one tissue homogenization and one Proteinase K digestion.

Protocols

GenePurgeDirect® Protocol for Mouse Tail with Tissue Homogenization Part I: Mouse Tail Homogenization

1. Cut a 1mm thick section from the either fresh or frozen mouse tail that has been rinsed with sterile water to remove any surface contamination and place the section into the bottom of a 1.5 ml tube.
2. Add 25µl of 1X TE to the tube containing the sectioned tail tissue.
3. Mince the section of tissue by thrusting a pestle against the tissue and twisting the pestle to compress the tissue against the walls of the tube. Ten thrusts with the pestle are sufficient.
4. Place the tube containing the homogenate at 4°C as each specimen is homogenized.

Part II: GenePurgeDirect® Treatment

5. Transfer 1µl of the tissue homogenate obtained above into a 0.5 ml standard amplification tube. NOTE: flick the tube containing the homogenate 3 times to mix before transfer.
6. Resuspend the GenePurgeDirect® mixture by vortexing 2-3 seconds or inverting 510 times.
7. Add 20ul of GenePurgeDirect® suspension to the 1ul of homogenate in the PCR tube and tightly close the tube lid.
8. Pulse vortex briefly to mix.
9. Place samples onto thermal cycler, with a heated lid, with the following GenePurgeDirect® lysis 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

10. Once program is completed, centrifuge sample tubes at 5,000 x g for 1 minute.
11. Remove supernatant to use as template for PCR. Recommend using 1-10ul of supernatant per 20-100ul amplification reaction. Alternatively, PCR can be performed directly in the GenePurgeDirect® treatment tube; add amplification reagents for a final volume of 100ul.
12. Perform amplification reaction according to your optimized protocol.

* GenePurgeDirect® treatment can alternatively be performed in a microwave, see page 2 for the protocol.

GenePurgeDirect® Protocol for Mouse Tail with Proteinase K Digestion

1. Cut a 1mm thick slice of mouse tail which has been washed with sterile water to remove surface contamination.
2. Place this section of tail in a 0.5ml PCR tube
3. Add 50µl 1XTE.
4. Resuspend the GenePurgeDirect® mixture by vortexing 2-3 seconds or inverting 5-10 times.
5. Add 20µl GenePurgeDirect®.
6. Add 2µl Proteinase K (conc.14-15 mg/ml) and mix well.
7. Digest on thermal cycler 1-3 hours @ 55°C.
8. Vortex to resuspend.
9. Heat inactivate 95°C for 10 minutes. (This is critical - DO NOT SHORTEN TIME OR LOWER TEMPERATURE - IF PK IS NOT COMPLETELY INACTIVATED IT WILL DIGEST TAQ.)
10. Centrifuge 5 minutes @ 10,000xg.

11. Carefully transfer supernatant to fresh 1.5ml sterile screw cap tube to use as template for PCR.
12. Use 1µl, 2.5µl, and 5µl of supernatant as template for 3-100µl reactions. This is a range finding step. Thereafter use whichever performed best.
13. 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 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!

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

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