LIFE TECH Life Technologies™
Your Molecular & Cell Technology Partner

NIMAGEN

GenePurgeDirect® DNA/RNA Releasing Agent

RT-PCR on GenePurgeDirect® Treated Samples

Version: 1.0
Revision date: 31-07-2014

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

Protocols

GenePurgeDirect® Protocol for RTs PCR Ready RNA

1. Place an aliquot of cells containing 10^6 cells/ml in a volume of 1-10µl into a standard amplification tube.
2. Thoroughly resuspend the contents of the GenePurgeDirect® tube by vortexing briefly.
3. Add 20µl of GenePurgeDirect® to the cells and vortex briefly for 1o 2 seconds.
4. Treat samples using either the thermal cycler program (below) or the microwave protocol (page2)
5. After the GenePurgeDirect® program is complete, add 0.1 to 1 unit of DNase (RNase free) to digest the DNA, follow the manufacturers recommended incubation and enzyme inactivation protocol.
6. Add 0.1 units of Proteinase K and heat to 55ºC for 30 minutes followed by a heat inactivation step at 95ºC for 3-10 minutes.
7. Centrifuge tubes at 10,000xg for 2 minutes to tightly pellet the GenePurgeDirect® resin.
8. Perform the Reverse Transcription (RT) reaction according to the manufacturers protocol, directly in the tube limiting the total volume of reagents to 50µl.
9. Following the RT reaction inactivate the enzyme per manufacturers protocol.
10. Perform PCR in the tube containing the cDNA produced by the RT reaction according to your optimized protocol.
    - Recommend a 100µl PCR reaction volume.
    - Buffers, magnesium, etc., may need to be adjusted so that the final PCR volume is 100µl and all components are at a 1X concentration.

GenePurgeDirect® Protocol for RTs PCR Ready RNA – without DNase/Proteinase K Treatment

1. Accomplish lysis in a standard amplification tube using either the thermal cycler or microwave GenePurgeDirect® protocol.
    - Example: 1µl whole blood added to 20 µl of GenePurgeDirect®.
2. Centrifuge the sample tube at 5,000xg for 1 minute and remove the supernatant to use as a template.
3. Take an aliquot of the supernatant and perform Reverse Transcription (RT) according to the manufacturers protocol.
4. Following the RT reaction inactivate the enzyme per manufacturers protocol and take a suitable aliquot of the RT reaction to use as a template for PCR. We recommend 1-10µl supernatant for a 20-100µl amplification reaction.
5. Perform amplification reaction according to your optimized protocol.

Thermal Cycler Lysis Protocol:

Place samples with GenePurgeDirect® 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.
9.	80ºC	hold

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.

References:

Parakh DR, Shamloul AM, Hadidi A, Waterworth HE, Scott SW, Howell HE, Mink GI. Detection of Prune Dwarf Ilarvirus from Infected Stone Fruits Using Reverse-Transcription-Polymerase Chain Reaction. XVI International Symposium on Fruit Tree Virus diseases. July 1995.

Shamloul AM, Hadidi A, Zhu SF, Singh RP, Sagredo B. Sensitive detection of potato spindle tuber viroid using RT-PCR and identification of an viroid variant naturally infecting pepino plants. Canadian Journal of Plant Pathology. 1997, 19(1):89-96.