

GenePurgeDirect® DNA/RNA Releasing Agent

Blood Serum Plasma

Version: 1.0

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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.

Protocols

GenePurgeDirect®!Protocol!for!Whole!Blood!

1. Place 1µl of whole blood into the bottom of a 0.2ml/0.5ml amplification tube that is PCR compatible with your specific thermal cycler.
2. Resuspend the GenePurgeDirect® mixture by vortexing 2-3 seconds or inverting 5-10 times.
3. Add 20µl of GenePurgeDirect® suspension to the 1µl of whole blood in the PCR tube and tightly close the tube lid. Pulse vortex briefly to mix.
4. Place samples onto thermal cycler, with a heated lid, with the following GenePurgeDirect® 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	

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 1E10µl of supernatant per 20E100µl amplification reaction. Alternatively, PCR can be performed directly in the GenePurgeDirect® treatment tube; add amplification reagents for a final volume of 100µl.
7. Perform amplification reaction according to your optimized protocol.
* GenePurgeDirect® treatment can alternatively be performed in a microwave, see page 3 for the protocol.

GenePurgeDirect®!Whole!Blood!with!Nucleated!Cellular!Enrichment!Protocol!

In order to perform DNA amplification to detect certain viruses or other intracellular organisms it is necessary to obtain the population of nucleated cells from whole blood. The following procedure provides a rapid method for obtaining a population of nucleated cells.

1. Transfer an aliquot of whole blood containing a population of 10⁶ nucleated cells (~200µl) to a 0.5µl amplification tube.
2. To the aliquot of whole blood add an equal volume of 1% Triton XE100 (1% wt/volume prepared in sterile H₂O).
3. Vortex 1E2 seconds and then place on ice or at 4°C for 5 minutes.
4. Remove the tubes from 4°C and centrifuge for 1 minute at 12,000xg.
5. Remove and discard the supernatant containing the RBC lysate.
6. Wash the remaining cell pellet 2 times with 200µl of 1X PCR buffer (which includes 1.5mM MgCl₂) by resuspending the cells in the buffer and repeating the centrifugation step.
7. Following the last wash step, discard the supernatant as completely as possible.
8. Resuspend the GenePurgeDirect® mixture by vortexing 2-3 seconds or inverting 5-10 times.
9. Add 20µl of GenePurgeDirect® and proceed with either the thermal cycler (above) or microwave lysis protocol (p3).
10. Once program is completed, centrifuge tubes at 5,000xg for 1 minute.
11. Transfer supernatant to a new tube for use as template for PCR. Recommend using 1E10µl of supernatant per 20E 100µl amplification reaction. Alternatively, 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.
* GenePurgeDirect® treatment can alternatively be performed in a microwave, see page 3 for the protocol.

GenePurgeDirect®!Whole!Blood!t !Multiple!Analysis!Protocol!

1. Collect whole blood by standard venipuncture or other techniques observing appropriate protection protocols. EDTA, Heparin and ACD preservatives are suitable.

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2. Transfer an aliquot of whole blood with a population of 10⁶ nucleated cells (approximately 200µl) to a 0.5ml amplification tube. (NOTE: DO NOT USE ANOTHER SIZE TUBE!).
3. Add to the whole blood aliquot an equal volume of 0.14M NH₄Cl, 0.017M TRIS, pH 7.4. Vortex this mix 5 seconds.
4. Place on ice or at 4°C for 5 minutes.
5. Remove tubes from ice. If variable speed centrifuge is used, centrifuge at 1,000xg for 1 minute. If fixed speed centrifuge is used, pulse in 3 second intervals for 12 seconds or 4X.
6. Remove and discard the supernatant containing the RBC lysate being careful not to dislodge the cell pellet.
7. Wash the remaining pellet 3 times with 200µl of 1X PCR buffer (which includes 1.5 mM MgCl₂). Vortex briefly and centrifuge as per steps 4 and 5 above and discard the supernatant.
8. Resuspend the GenePurgeDirect® mixture by vortexing 20 3 seconds or inverting 50 10 times
9. To the remaining pellet, add 20µl 1XTE and 20µl GenePurgeDirect®.
10. Proceed with either the thermal cycler or microwave lysis protocols.
11. Once lysis protocol is complete, centrifuge 30 seconds, draw off 20µl of the supernatant which now contains the DNA/RNA released from cells, and transfer this to a fresh tube and bring up to a total volume of ~200µl with the addition of 180µl of 1XTE.
12. The supernatant is now suitable for use as template for PCR. Use 1E10µl of supernatant per 20E100µl amplification reaction. Use for PCR or store at 4°C for later PCR. Amplification can be successfully performed on specimens processed by this protocol and stored at 4°C for over 60 days. Data suggests that specimens processed as per above can be amplified after at least one year when stored at E20°C.
13. Perform amplification reaction according to your optimized protocol.

GenePurgeDirect®!Serum!and/or!Plasma!Protocol!

1. Obtain plasma or serum by standard collection techniques.
 2. Resuspend the GenePurgeDirect® mixture by vortexing 2-3 seconds or inverting 5-10 times.
 3. For maximum sensitivity, treat 5µl of serum with 15µl of resuspended GenePurgeDirect®.
 4. Vortex the mixture briefly for 1E2 seconds.
 5. Proceed with either the thermal cycler or microwave lysis protocol.
 6. When samples are ready to use place in a thermal cycler and heat to 80°C for 5 minutes.
 7. The sample is now ready to use as a PCR template.
 8. Add appropriate volume of mastermix to the template and perform amplification reaction according to your optimized protocol.
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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
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 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:

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