

Introduction

PhaseShield™ Gel (PLG) is a proprietary tool for avoiding contamination of the protein layer when extracting DNA or RNA with organic reagents. PLG shortens the extraction operation time while increasing the nucleic acid yield. Under the action of centrifuge, PLG can form a dense stationary phase between the water phase and the organic phase, and the substances in the organic phase are effectively isolated below PLG. The formation of a dense solid-phase layer allows the experimenter to easily transfer nucleic acids from the aqueous phase into clean tubes.

When PLG is used in nucleic acid extraction experiment, the yield of nucleic acid can be increased by 10%-20%, which can effectively avoid the contact between experimenters and toxic substances, and there is no need to worry about whether the sample will be contaminated when extracting. PLG can be applied to the experimental operation of liquid phase extraction with any organic reagent (phenol or chloroform).

Package Information

Components	M2302
PhaseShield™ Gel Tubes, Heavy	2 ml/15 ml/50 ml

Storage

Store at room temperature and cannot be frozen at low temperature.

Applications

1. Recovery of DNA from agarose gel electrophoresis.
2. M13/Phagemid DNA Separation and Purification.
3. Isolation and purification of Lambda DNA.
4. Genomic DNA extraction from whole blood.
5. Extraction of DNA from cells in tissue culture.
6. Extraction of genomic DNA from the mouse tail.
7. Extraction of plasmid DNA by traditional method.
8. Extraction of total RNA from various samples.

Protocol

1. Before use, put PLG in a centrifuge and centrifuge for 20-30 seconds at 12,000-16,000× g.
2. Add 100-750 μl (PLG 2 ml) liquid sample and the same volume of organic extraction reagent to PLG.
3. Thoroughly mix the organic and aqueous phases to form a mixed phase solution.

Note: No vortex mixing.

4. Centrifuge the mixed solution at 12,000-16,000× g for 5 minutes, so that it can be separated. PLG will form a dense solid layer between the organic phase and the water phase.

A small amount of PLG may remain at the bottom of the centrifuge tube, which will not affect the effect. If secondary extraction is needed, the mixed solution can be added to the tube (the upper layer of the layered PLG) without exceeding the volume limit of the centrifuge tube.

5. Pour it directly or pipette to carefully transfer the upper PLG aqueous phase containing nucleic acid to another clean centrifuge tube.

6. Add salt solution or alcohol to the transferred aqueous phase to precipitate nucleic acid (nucleic acid precipitation aid can also be added if necessary), and carry out subsequent routine experimental operations.

Supplementary operation flow of extracting total RNA with PLG (Heavy) and TRNzol series reagents

Prepare reagents: Chloroform/isopropanol/RNase-Free ddH₂O/75% ethanol (prepared with RNase-Free ddH₂O)

1. Homogenization

a. Plant tissue: Take fresh leaves and grind fully in liquid nitrogen or cut into pieces and grind directly in TRNzol series reagents. Grinding should be fast, preferably not more than 1 minute. Use 1 ml TRNzol series reagents for about 100 mg leaves.

b. Animal tissue: Take fresh tissues or tissues frozen at -70°C, add 1 ml TRNzol series reagents to every 30-50 mg of tissues, and homogenize with homogenizer. The sample volume should generally not exceed 10% of the volume of TRNzol series reagents.

c. Cell suspension: centrifuge the cells. Add 1 ml TRNzol series reagents to every 5×10⁶-10⁷ cells. Do not wash the cells before adding TRNzol series reagents to avoid degradation of mRNA.

d. Blood treatment: directly take fresh blood, add 3 times the volume of TRNzol series reagents (it is recommended to add 0.75 ml TRNzol series reagents to 0.25 ml of whole blood), and fully shake and mix.

2. Let the homogenized sample stand at 15-25°C for 5 minutes.

3. Put the PLG (Heavy) into a centrifuge and centrifuge for 20-30 seconds at 12,000-16,000× g.

4. Transfer all homogenized samples in step 2 to PLG after pre-centrifugation.

a. Add 0.2 ml chloroform to 1 ml TRNzol series reagents, cover the tube and shake violently for 15 seconds.

b. Add 100 μl RNase-Free ddH₂O and 0.2 ml chloroform to every 1 ml TRNzol series reagents, cover the tube and shake violently for 15 seconds.

Note: No vortex mixing.

5. Leave the sample at room temperature for 2-3 minutes.

6. Centrifuge at 4°C and 12,000× g for 15 minutes. PLG will form a dense solid layer between organic phase and water phase.

7. Transfer the aqueous phase containing RNA on the upper layer of PLG to another RNase-Free centrifuge tube.

Note: If the sample volume after homogenization in step 2 is large, it can be operated in stages. After transferring the upper water phase, add the remaining homogenized sample into PLG tube with lower organic phase and solid layer, repeat steps 4, 5 and 6, 7, and combine the water phases.

8. Adding equal volume of isopropanol into the obtained aqueous solution, thoroughly mixing, and standing at room temperature for 20-30 minutes.

9. Centrifuge at 4°C and 12,000× g for 10 minutes, and remove the supernatant.

10. At least 1 ml of 75% ethanol (prepared with RNase-Free ddH₂O) is added for every 1 ml of TRNzol series reagents, and the precipitate is washed.

11. Centrifuge at 4°C and 7500× g for 5 minutes. Pour out the liquid without disturb the precipitate.

12. Leave it to dry at room temperature about 2-3 minutes (don't dry it too much, otherwise dissolve RNA will be difficult), according to the needs of the experiment, add 30-100 µl of RNase-Free ddH₂O. Pipette and mix it well to fully dissolve the RNA.

Note: TRNzol series reagents include TRNzol, TRNzol A+, and TRNzol Universal. If it is used with other brands of RNA extraction reagents, please refer to the operation flow of PLG and TRNzol or refer to the detailed instructions.