LIFE SCB
LIFE SCIENCE BIOTECHNOLOGY

Email: info@lifescb.com
Tel: +1-301-890-6420 Fax: +1-301-965-8269
9610 Medical Center Drive, Rockville, MD 20850, USA

PhaseShield™ Gel Tubes

Cat. #: M2302 Size: 2 ml/15 ml/50 ml

Introduction

PhaseShield™ Gel (PLG) is a proprietary reagent designed to prevent protein contamination during organic nucleic acid extractions. By forming a dense, stable barrier between the aqueous and organic phases during centrifugation, PLG effectively sequesters proteins and organic solvents. This solid-phase layer allows researchers to easily and completely recover the nucleic acid-rich aqueous phase without the risk of carryover. Implementing PLG can increase nucleic acid yields by 10–20% while significantly reducing exposure to hazardous chemicals. Compatible with any phenol-chloroform protocol, PhaseShield™ Gel streamlines the extraction process without compromising purity.

Package Information

Components	M2302
PhaseShield™ Gel Tubes	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

PhaseShield™ Gel 2 ml General Protocol

1. Immediately prior to use, spin the PhaseShield™ Gel tubes at 12,000 x g in a microcentrifuge for 20-30 seconds.

2. Add 100 to 750 µL of aqueous sample and an equal volume of organic extraction reagent directly to a pre-spun PhaseShield™ Gel tube.

3. Mix the organic and aqueous phases thoroughly by vigorous shaking to form a transiently homogeneous suspension (Do not vortex).

4. Centrifuge at 12,000 x g for 5 minutes to separate the phases. The PhaseShield™ Gel should form a durable and intact barrier between the aqueous and organic phases effectively separating and preventing the organic phase and interface material from contaminating the aqueous phase. A small amount of PhaseShield™ Gel may remain in the bottom of the tube. A second extraction can be performed in the same tube by adding more organic extraction reagent, mixing and re-centrifuging the tube.

5. Carefully pipet or decant the nucleic-acid-containing aqueous phase (upper layer) to a fresh tube.
6. Precipitate the nucleic acid with the addition of salt and alcohol according to application-specific protocols.

PhaseShield™ Gel 15 ml and 50 ml General Protocol

1. Immediately prior to use, spin the PhaseShield™ Gel tubes at 1,500× g in a microcentrifuge for 1-2 min.

2. Add 1-6 ml (15), or 5-20 ml (50) of aqueous sample and an equal volume of organic extraction reagent directly to a pre-spun PhaseShield™ Gel tube.

3. Mix the organic and aqueous phases thoroughly by vigorous shaking to form a transiently homogeneous suspension (Do not vortex).

4. Centrifuge at 1,500× g for 2 minutes to separate the phases. The PhaseShield™ Gel should form a durable and intact barrier between the aqueous and organic phases effectively separating and preventing the organic phase and interface material from contaminating the aqueous phase. A small amount of PhaseShield™ Gel may remain in the bottom of the tube. A second extraction can be performed in the same tube by adding more organic extraction reagent, mixing and re-centrifuging the tube.

5. Carefully pipet or decant the nucleic-acid-containing aqueous phase (upper layer) to a fresh tube.

6. Precipitate the nucleic acid with the addition of salt and alcohol according to application-specific protocols.

RNA Isolation Using TRIzol Reagent or Similar Products with PhaseShield™ Gel Heavy

1. Homogenization

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

1.2 Animal tissue: Take fresh tissues or frozen tissues, add 1 ml TRIzol reagent to every 30-50 mg of tissues, and homogenize with homogenizer. The sample volume should generally not exceed 10% of the volume of TRIzol reagent.

1.3 Cell suspension: Centrifuge the cells. Add 1 ml TRIzol reagent to every 5×10^{6}-10^{7} cells. Do not wash the cells before adding TRIzol reagent to avoid degradation of mRNA.

1.4 Blood: Directly take fresh blood, add 3 times the volume of TRIzol reagent and fully shake and mix.

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

3. Spin the PhaseShield™ Gel tubes at 12,000× g in a microcentrifuge for 20-30 seconds.

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4. Transfer all homogenized samples in step 2 to PhaseShield™ Gel tube.

4.1 Add 0.2 ml chloroform per ml of lysis reagent, cover the tube and shake violently for 15 seconds.

Note: No vortex mixing.

4.2 Add 0.1 ml RNase-Free ddH_2O and 0.2 ml chloroform per ml of lysis reagent, cover the tube and shake violently for 15 seconds.

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

6. Centrifuge at 4°C and 12,000× g for 5 minutes. PhaseShield™ Gel will form a dense solid layer between organic and aqueous phase.

7. Transfer the aqueous phase containing RNA on the upper layer 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 Phase Lock Gel tube with lower organic phase and solid layer, repeat steps 4, 5, 6 and 7, and combine the aqueous 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. Add 1 ml of 75% ethanol (prepared with RNase-Free ddH_2O) for every 1 ml of lysis reagent, 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, according to the needs of the experiment, add 30-100 µl of RNase-Free ddH_2O. Pipette and mix it well to fully dissolve the RNA.

Troubleshooting

Phase Lock Gel is not phasing properly

1. Wrong Phase Lock Gel type（Heavy or Light)

Check the compatibility chart and choose the correct gel type

2. The centrifuge speed was not correct

Check the protocol to assuret he centrifuge speed is correct

3. Phase Lock Gel was not spun down prior touse

Spin Phase Lock Gel down prior to use

Phase Lock Gel migrates above theaqueous phase

1. The aqueous phase is too dense

Pierce the Phase Lock Gel barrier with a pipette tip, add water or buffer to lower the density of the aqueous phase, mix and re-spin the tube

Phase Lock Gel remains at thebottom of the tube

1. The organic phase is not dense enough

Add chloroform to increase the density of the organic phase, mix and re-spin the tube

Phase Lock Gel is not phasing properly with samples in TRIzol Reagent or similar product

1. Aqueous phase is too dense

If the Phase Lock Gel barrier is intact and the aqueous layer can be removed completely by pipetting or decanting, proceed with the protocol

Add RNAse-free water or buffer (up to 0.2 ml per 1 ml of lysis reagent) to lower the density of the aqueous phase, mix and re-spin the tube