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Phase Lock Gel, Heavy and Light

Catalog Number	Type	Packaging Size	Sample Volume
FP323-1	2 mL, Heavy	1×50	100-750 µL
FP323-2	2 mL, Heavy	2×50	100-750 µL
FP323-3	2 mL, Heavy	4×50	100-750 µL
FP324-1	2 mL, Light	1×50	100-750 µL
FP324-2	2 mL, Light	2×50	100-750 µL
FP324-3	2 mL, Light	4×50	100-750 µL
FP325	15 mL, Heavy	40	1-6 mL
FP326	15 mL, Light	40	1-6 mL
FP327	50 mL, Heavy	15	15-20 mL
FP328	50 mL, Light	15	15-20 mL

Store at Room Temperature — DO NOT FREEZE!

Product Description

Phase Lock Gel (PLG) is a unique product that eliminates interphase-protein contamination during organic extraction and ensures faster results with improved recoveries. Phase Lock Gel, when used during phenol, phenol:chloroform, or phenol:chloroform:isoamyl alcohol extractions, migrates under centrifuge force to form a tight seal between the aqueous and organic phase. The organic phase and the interphase materials are effectively trapped below the Phase Lock Gel barrier, thus enabling complete and easy decanting or pipetting of the entire aqueous phase. The benefits are increased yields of up to 30%, increased protection from exposure to hazardous compounds, and no risk of interphase sample contamination.

Phase Lock Gel is inert, stable to heating and does not interfere with standard nucleic acid restriction and modification enzymes. In fact, many of the reactions can be carried out in the presence of Phase Lock Gel at the appropriate temperature and then terminated by the addition of phenol or phenol:chloroform. The nucleic acid can then be extracted by following standard protocols. Phase Lock Gel can be present during the heat inactivation of enzymes (65°C for 10 minutes) prior to organic extraction.

Applications and Compatibility

Phase Lock Gel Heavy and Light can be used with virtually any protocol where an aqueous sample is extracted with phenol, phenol-chloroform or chloroform. For optimum phase separation the Phase Lock Gel barrier must have a lower density than the organic phase and a higher density than the aqueous phase. Phase Lock Gel Light is required for samples extracted with phenol. Phase Lock Gel Heavy is not compatible with samples extracted with phenol because Phase Lock Gel Heavy has a higher density than phenol. Either Phase Lock Gel Heavy or Light can be used for samples extracted with phenol-chloroform or chloroform. Phase Lock Gel Heavy is required for samples with high salt or high protein concentrations (>0.5M or >1 mg/mL, respectively) since salt and proteins can increase the density of the aqueous phase to a density higher than Phase Lock Gel Light. Please consult the Organic Phase Compatibility table below for the formulation that fits your application. If phase separation is sub-optimal, the sample can be adjusted by the addition of water or buffer to make the aqueous phase lighter or by the addition of chloroform to make the organic phase heavier.

Organic Phase Compatibility

Aqueous Phase	Organic Phase			
	Phenol:Chloroform: Isoamy alcohol (25:24:1)	Chloroform: Isoamy alcohol (24:1)	H_2O or Buffer-saturated Phenol:Chloroform (1:1)	H_2O or Buffer-saturated Phenol
<0.5 M NaCl	Heavy, Light	Heavy, Light	Heavy, Light	Light
<1 mg/mL BSA	Heavy, Light	Heavy, Light	Heavy, Light	Light
Genomic DNA isolation	Heavy, Light	Heavy, Light	Heavy, Light	Light
Plasmid DNA isolation	Heavy	Heavy	Heavy	Not compatible
RNA isolation	Heavy	Heavy	Heavy	Not compatible

Protocol

A. Phase Lock Gel 2 mL General Protocol

1. Immediately prior to use, spin the Phase Lock 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 Phase Lock 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 Phase Lock 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 Phase Lock 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.

B. Phase Lock Gel 15 and 50 mL General Protocol

1. Immediately prior to use, spin the Phase Lock Gel tubes at 1,500 x 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 Phase Lock 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 x g for 2 minutes to separate the phases. The Phase Lock 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 Phase Lock 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.

C. RNA Isolation Using TRIzol Reagent or Similar Products with Phase Lock Gel Heavy

1. Homogenization
   a. 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.

b. 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.

c. 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.

d. 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 Phase Lock Gel tubes at 12,000 x g in a microcentrifuge for 20-30 seconds.
4. Transfer all homogenized samples in step 2 to Phase Lock Gel tube.
   a. Add 0.2 mL chloroform per mL of lysis reagent, cover the tube and shake violently for 15 seconds.
   b. 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.

   Attention: No vortex mixing.
5. Leave the sample at room temperature for 2-3 minutes.
6. Centrifuge at 4°C and 12,000×g for 5 minutes. Phase Lock 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.

   Attention: 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

Problem	Possible Cause	Resolution
Phase Lock Gel is not phasing properly	Wrong Phase Lock Gel type (Heavy or Light)	Check the compatibility chart and choose the correct Phase Lock Gel type (Heavy or Light)
	The centrifuge speed was not correct	Check the protocol to assure the centrifuge speed is correct
	Phase Lock Gel was not spun down prior to use	Spin Phase Lock Gel down prior to use
Phase Lock Gel migrates above the aqueous phase	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 the bottom of the tube	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	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