5PRIME Phase Lock Gel™ – Heavy and Light

Cat. No. 2302830– Heavy / 200 x 2.0 mL Tubes
2302820 – Light / 200 x 2.0 mL Tubes

Store at Room Temperature
DO NOT FREEZE

Description

Extraction of nucleic acids with phenol and chloroform is a standard molecular biology technique widely used in methods designed to isolate and purify DNA and RNA. After extraction and centrifugation, nucleic acids remain in the aqueous phase (upper layer) while proteins partition into the organic phase (lower layer) or lie at the phase interface. A major drawback of the technique is that it is often very difficult and cumbersome to completely recover the aqueous phase without disturbing the interface between the two phases. 5PRIME Phase Lock Gel forms a durable barrier separating the aqueous phase from the organic phase. Since the organic phase and interface material are effectively trapped below the Phase Lock Gel barrier, the aqueous phase can be much more easily and completely recovered by pipetting or decanting without risk of contamination with the organic phase or material at the interface. The use of Phase Lock Gel thus affords the researcher an easier process that produces an increased recovery of nucleic acid samples with higher purity and a reduced risk of exposure to hazardous chemicals.

Components

Catalog Number	Type	Sample Volume	Tube Color	Properties
2302830	2 mL, Heavy	100 -750 µL	Yellow	Opaque
2302820	2 mL, Light	100 -750 µL	Green	Translucent

Storage and Stability

5PRIME Phase Lock Gel is stable when stored at room temperature (**DO NOT FREEZE**). Phase Lock Gel is inert and does not interfere with standard nucleic acid restriction and modification enzymes. Phase Lock Gel can be present during the heat inactivation of enzymes (65°C for 10 minutes) prior to organic extraction. For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

Applications and Compatibility

5PRIME 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 (see the **Troubleshooting** table).

Organic Phase Compatibility

Aqueous phase	Organic Phase			
	PCI	CI	H_2O or Trisbuffer saturated PC	H_2O or Trisbuffer saturated phenol
< 0.5 M NaCl[a]	H, L	H, L	H, L	L
< 1 mg/ml BSA[a]	H, L	H, L	H, L	L
Plasmid DNA isolatio[b]	H	H	H	O
Genomic DNA isolation[c]	H, L	H, L	H, L	L
RNA isolation[d]	H	H	H	-

L = Light, H = Heavy ,- = This combination of aqueous and organic phases is not suitable for use with 5PRIME Phase Lock Gel.

a. Preparation of total RNA using guanidinium isothiocyanate in conjunction with organic extraction.[1] Phase Lock Gel Heavy can be used with TRIzol® Reagent or similar products with modifications (see **Note** in Step 2 of the **Protocol** section).

b. Bacterial cleared lysates prepared according to the alkaline lysis procedure.[2]

c. Protocols in which nuclei are first pelleted from cells lysed in the presence of 1% Triton X-100, and then lysed in saline/EDTA and SDS with or without the addition of Proteinase K.[3]

d. For optimum results with Phase Lock Gel Light, the starting sample should not exceed 0.5 M NaCl or 1 mg/mL protein. Samples exceeding these concentrations should be diluted prior to extraction. If dilution is inappropriate, extractions may be performed with PS Heavy in combination with phenol-chloroform or chloroform. Phase Lock Gel Heavy is not compatible with phenol extractions as shown in the table.

Precautions and Warnings

This product is used with hazardous chemicals that should be handled, stored and disposed of according to the manufacturer's instructions.

Protocol

1. Immediately prior to use, spin the 5PRIME Phase Lock Gel tubes at 12,000 x g in a microcentrifuge for 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. Mix the organic and aqueous phases thoroughly by vigorous shaking to form a transiently homogeneous suspension (Do not vortex).

   Note: For RNA isolation using TRIzol® Reagent or similar products add 0.5 to 1.25 mL of homogenized cell or tissue lysate to a pre-spun Phase Lock Gel tube and incubate for 5 minutes at room temperature. To ensure proper phase separation the addition of RNAse-free water or buffer (up to 0.2 mL per mL of lysis reagent) may be required to reduce the density of the aqueous phase. Add chloroform (0.2 mL per mL of lysis reagent) and shake vigorously by hand for 15 seconds to ensure thorough mixing (Do not vortex). Incubate for 2 minutes at room temperature and centrifuge at 12,000 x g for 15 minutes at 4 ˚C to separate the phases. Proceed to step 4 below. 1-bromo-3-chloropropane (0.1 mL per mL of lysis reagent) can be used instead of chloroform to minimize DNA contamination. [4]
3. 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.
4. Carefully pipet or decant the nucleic-acid-containing aqueous phase (upper layer) to a fresh tube.
5. Precipitate the nucleic acid with the addition of salt and alcohol according to application-specific protocols.

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 may have been frozen	Store Phase Lock Gel at room temperature (**DO NOT FREEZE**)
	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

References

1. Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162:156-159.
2. Birnboim, H.C. and Doly, J. (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucl. Acids Res. 7:1513-1522.
3. Herrmann, B.G. and Frischauf, A.M. (1987) Isolation of genomic DNA. Methods Enzymol. 152:180-183.
4. Chomczynski, P. and Mackey, K. (1995) Substitution of chloroform by bromochloropropane in the single-step method of RNA isolation. Anal. Biochem. 225:13-164.

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