

Product Information

Phase Divider Gel, Light And Heavy

Catalog Numbers **P1723, P1848, P1973, P2098, P2223, P2348, P2473, P2598, P2723**

	Product Number	Tube Size	Sample Size
Light	P 1723	1.5 ml	50-500 µl
	P 2098	15 ml	1-6 ml
	P 2348	50 ml	5-20 ml
	P 2598	3 ml syringe	Custom
Heavy	P 1848	1.5 ml	50-500 µl
	P 1973	10 ml	1-4 ml
	P 2223	15 ml	1-6 ml
	P 2473	50 ml	5-20 ml
	P 2723	3 ml syringe	Custom

Product Description

After organic extraction, it is often difficult to recover nucleic acid in the aqueous upper phase free from the denatured protein at the aqueous and organic phase interface. Phase Divider Gel, when used during phenol, phenol:chloroform, or phenol:chloroform:isoamyl alcohol extractions, migrates under centrifugal force to form a seal between the organic and aqueous phases. The organic phase and the interface material are effectively trapped below the Phase Divider Gel. The barrier is sufficiently durable that the aqueous upper phase, containing the nucleic acid, can then be recovered quantitatively by simply decanting or pipetting to a fresh tube.

Phase Divider 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 Divider 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 Divider Gel does not interfere with the heat inactivation of enzymes (65°C for 10 minutes) prior to the organic extraction. Note that Phase Divider Gel is **not suitable** for use with Tri Reagent (Product No. T9424).

Phase Divider Light Gel can be used to improve the recovery of DNA fragments from low melting point agarose with only minor changes to the standard protocol. It may also be used in the standard protocols for the preparation of plasmid DNA from *E. coli*, phagemid DNA from M13-type phage, and phage DNA from lambda. Phase Divider Light Gel also may be used for isolating high molecular weight genomic DNA from blood, cultured cells, and tissue. The use of Phase Divider Heavy Gel in a microprep plasmid DNA purification protocol allowed for the direct screening of inserts as well as probe synthesis and/or DNA sequencing within one day. Phase Divider Heavy Gel may be used to prepare partially purified plasmid DNA from *E. coli* and for the preparation of total RNA by homogenization in guanidine thiocyanate and organic extraction.

The ability of Phase Divider Gel to separate the phases is dependent upon the composition of the aqueous and the organic media. Salt and protein in the aqueous phase have an effect on both the aqueous and organic phase densities, while different organic phase formulations also vary in density. For optimum phase separation, the compositions of the aqueous phase, organic phase, and Phase Divider Gel must be

compatible. As a result, Phase Divider Gel is offered in two different density formulations, Light and Heavy. Please consult the following table for the formulation which fits the desired application.

Applications and Compatibility

Choosing the correct density of Phase Divider Gel for a system is crucial. Consult the table below to select the correct density for the specific application.

Aqueous phase	Organic phase			
	Phenol:Chloroform: Isoamy alcohol (25:24:1)	Chloroform: Isoamyl alcohol (24:1)	H ₂ O or Buffer- saturated Phenol: Chloroform (1:1)	H ₂ O or Buffer- saturated phenol
≤0.5 M NaCl	Light, Heavy	Light, Heavy	Light, Heavy	Light
≤1 mg/ml BSA	Light, Heavy	Light, Heavy	Light, Heavy	Light
Cleared lysate	Heavy	Heavy	Heavy	Not compatible*
Homogenized tissue	Light, Heavy	Light, Heavy	Light, Heavy	Light
RNA purification	Heavy	Heavy	Heavy	Not compatible*

* This combination of aqueous and organic phases is not suitable for use with Phase Divider Gel. Note that Phase Divider Gel is **not suitable** for use with Tri Reagent.

NOTE: For optimum results with Phase Divider Light Gel, 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 Phase Divider Heavy Gel in combination with phenol:chloroform:isoamyl alcohol (25:24:1), phenol:chloroform (1:1), or chloroform:isoamyl alcohol (24:1). Phase Divider Heavy Gel is not compatible with water or buffer-saturated phenol as shown in the table.

Storage

Store Phase Divider Gel at room temperature.

Do not freeze.

Usage Note

Phase Divider tubes should be centrifuged immediately prior to adding the aqueous and/or organic phases to them.

A. Phase Divider Gel 1.5 General Protocol

Immediately prior to use, pellet the Phase Divider Gel at full speed in a microcentrifuge for 20-30 seconds.

1. Add 100-500 µl of aqueous sample and an equal volume of organic extraction solvent directly to the 1.5 ml microcentrifuge tube containing Phase Divider Gel.
2. Thoroughly mix the organic and aqueous phases to form a transiently homogeneous suspension. The Phase Divider Gel will not become part of the suspension. **DO NOT VORTEX.**
3. Centrifuge at full speed (12,000x g or greater in a microcentrifuge) for 2 minutes to separate the phases. The Phase Divider Gel will form a barrier between the aqueous and organic phases. A small amount of Phase Divider Gel may remain in the bottom of the tube.
4. Carefully decant, or transfer by pipette, the aqueous upper phase containing the nucleic acid to a fresh tube.

- Precipitate the nucleic acid by adding salt, alcohol and carrier (if needed) as per your protocol and applications.

B. Phase Divider Gel 10, 15 and 50 General Protocol

Immediately prior to use, pellet the Phase Divider Gel by centrifugation at 1500x g for 1-2 minutes.

- Add 1-4 ml (10), 1-6 ml (15), or 5-20 ml (50) of aqueous sample and an equal volume of organic extraction solvent directly to the centrifuge tube containing the Phase Divider Gel.
- Thoroughly mix the organic and aqueous phases by repeated inversion to form a transiently homogenous suspension. The Phase Divider Gel will not become part of the aqueous suspension. **DO NOT VORTEX.**
- Centrifuge for 2 minutes at 1500x g to separate the aqueous and organic phases (Phase Divider Gel 10 tubes may be centrifuged at up to 12,000x g.) The Phase Divider Gel will form a barrier between the aqueous and organic phases. A small amount of Phase Divider Gel may remain in the bottom of the tube.
- Carefully decant, or transfer by pipette, the aqueous upper phase containing the nucleic acid to a fresh tube.
- Precipitate the nucleic acid by adding salt, alcohol and carrier (if needed) as per your protocol and application.

C. Phase Divider Syringe General Protocol

- Without putting pressure on the plunger, twist off the orange cap and discard. Screw on the supplied gray dispensing tip to the syringe and tighten securely.
- Apply firm pressure on the plunger to dispense the Phase Divider Gel.
- The following amounts of Phase Divider Gel are recommended for optimum phase separation:

Approximately 100 μ l Phase Divider Gel for 0.65 ml microcentrifuge tubes.

Approximately 300 μ l Phase Divider Gel for 1.5 ml microcentrifuge tubes.

Approximately 2 ml Phase Divider Gel for disposable 15 ml screw-cap centrifuge tubes.

Approximately 5 ml Phase Divider Gel for disposable 50 ml screw-cap centrifuge tubes.

- Store the assembled syringe by pulling back on the plunger slightly. It is recommended that the gray tip be left in place, as Phase Divider Gel is quite slippery and can prevent adequate securing of the tip in the future.

Recovery of DNA from Low Melting Point Agarose

- Resolve the DNA fragments on a low melting point agarose (Product No. A 9414) in 1X Tris-Acetate-EDTA (TAE) buffer. **Do not use TBE buffer as borate-gels are much more difficult to dissolve.**
- Stain the gel with ethidium bromide. Visualize with a longwave UV light and carefully cut out the band(s) of interest with a sharp razor blade. (NOTE: Wear gloves when handling ethidium bromide and stained gels.)
- Transfer slice to a pre-spun (12,000x g for 20-30 seconds), pre-weighed Phase Divider Light Gel 1.5 tube and determine the weight of the slice.
- Add a volume (in μ l) of 1X TE, pH 8.0 (10 mM Tris-HCl, 1 mM EDTA, pH 8.0, Product No. T9285, diluted to working concentration) equivalent to 5X the weight (in mg) of the slice. Melt the slice in the TE at 65 °C for 5-10 minutes.
- Mix well to ensure the agarose slice is fully dissolved. Allow the dissolved sample to come to room temperature, and add an equal volume of room temperature buffer-saturated phenol, pH 8.0 (Product No. P 4557) to the sample. Thoroughly mix the organic and aqueous phases by repeated inversion to form a transiently homogenous suspension. The Phase Divider Gel will not become part of the aqueous suspension. **DO NOT VORTEX.**
- Centrifuge at full speed (12,000x g or greater in a microcentrifuge) for 2 minutes to separate the phases. Note: If the resulting aqueous phase still appears cloudy, the extraction with room temperature buffer-saturated phenol, pH 8.0, may be repeated.
- Recover the aqueous phase to a fresh Phase Divider Light Gel 1.5 tube and extract with an equivalent volume of room temperature phenol-chloroform-isoamyl alcohol (PCI, 25:24:1, Product No. P 3803 or P 2069). **DO NOT VORTEX.**
- Centrifuge at full speed (12,000x g or greater in a microcentrifuge) for 2 minutes to separate the phases. Recover the aqueous phase to a fresh Phase Divider Light Gel 1.5 tube, and extract with an equivalent volume of room temperature chloroform-isoamyl alcohol (CI, 24:1, Product No. C 0549).
- Centrifuge at full speed (12,000x g or greater in a microcentrifuge) for 2 minutes to separate the phases and transfer the aqueous phase to a suitably sized microcentrifuge tube.
- Add 0.25 volume of 10 M ammonium acetate and 2.5 volume of 100% ethanol to the sample and mix well.

11. Incubate at room temperature for 20 minutes, pellet by centrifugation, wash the pellet several times with cold 70% ethanol, air-dry the pellet and resuspend in a suitable buffer.

General References

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AH,RS,PHC 08/10-1