

## Product Information

### PHASE DIVIDER GEL LIGHT AND HEAVY

	Product Number	Tube Size	Sample Size
Light	P 2098	15 ml	1-6 ml
	P 2348	50 ml	5-20 ml
	P 2598	3 ml syringe	Custom
Heavy	P 2223	15 ml	1-6 ml
	P 2723	3 ml syringe	Custom

## TECHNICAL BULLETIN

### 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 organic extraction. Note that Phase Divider Gel is not suitable for use with TriReagent® (Product Number T 9424).

Phase Divider Light Gel can be used to improve the recovery of DNA 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.

### Precautions and Disclaimer

Phase Divider Gels are for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Storage/Stability

Store Phase Divider Gels at room temperature.

**Do not freeze.**

### 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:Isoamyl alcohol (25:24:1)	Chloroform:Isoamyl alcohol (24:1)	Water or Buffer-saturated Phenol/Chloroform (1:1)	Water 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 for 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.

### Procedure

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.

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

#### A. Phase Divider Gel in 15 and 50 ml tubes: General Protocol

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

1. Add 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 Phase Divider Gel.
2. Thoroughly mix the organic and aqueous phases by repeated inversion to form a transiently homogeneous suspension. The Phase Divider Gel will not become part of the suspension. **DO NOT VORTEX.**
3. Centrifuge for two minutes at 1500 x g full speed to separate the aqueous and organic 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.

5. Precipitate the nucleic acid by adding salt, alcohol, and carrier (if needed) as specified in your protocol or application.

**B. Phase Divider in Syringe: General Protocol**

1. 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.
2. Apply firm pressure on the plunger to dispense the Phase Divider Gel.
3. The following amounts of Phase Divider Gel are recommended for optimum phase separation.
  - a. Approximately 100  $\mu$ l Phase Divider Gel for 0.65 ml microcentrifuge tubes.
  - b. Approximately 300  $\mu$ l Phase Divider Gel for 1.5 ml microcentrifuge tubes.
  - c. Approximately 2 ml Phase Divider Gel for disposable 15-ml screw-cap centrifuge tubes.
  - d. Approximately 5 ml Phase Divider Gel for disposable 50-ml screw-cap centrifuge tubes.
4. 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.

**C. Recovery of DNA from Low Melting Point Agarose**

1. Resolve the DNA fragments on a low melting point agarose (Product Number A 9414) in 1x Tris-Acetate-EDTA (TAE) buffer. **Do not use TBE buffer as borate gels are much more difficult to dissolve.**
2. Stain the gel with ethidium bromide. Visualize with a long wave 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.)
3. Transfer the gel section to a pre-spun (12,000 x g for 20-30 seconds), pre-weighed Phase Divider Light Gel in a 1.5 ml tube and determine the weight of the slice.

4. Add a volume (in  $\mu$ l) of 1x TE, pH 8.0 (10 mM Tris-HCl, 1 mM EDTA, pH 8.0, Product Number T 9285, 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.

5. Mix well to ensure the agarose slice is fully melted. Allow the melted sample to come to room temperature, and add an equal volume of room temperature buffer-saturated phenol, pH 8.0 (Product Number P 4557), to the sample. Thoroughly mix the organic and aqueous phases by repeated inversion to form a transiently homogeneous suspension. The Phase Divider Gel will not become part of the aqueous suspension. **DO NOT VORTEX.**

6. Centrifuge at full speed (12,000 x g or greater in a microcentrifuge) for two 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.

7. Transfer the aqueous phase to fresh Phase Divider Light Gel in a 1.5 ml tube and extract with an equivalent volume of room temperature phenol-chloroform-isoamyl alcohol (PCI, 25:24:1, Product Number p 3803 or P 2069). **DO NOT VORTEX.**
8. Centrifuge at full speed (12,000 x g or greater in a microcentrifuge) for two minutes to separate the phases. Transfer the aqueous phase to fresh Phase Divider Light Gel in a 1.5 ml tube and extract with an equivalent volume of room temperature chloroform-isoamyl alcohol (CI, 24:1, Product Number C 0549).
9. Centrifuge at full speed (12,000 x g or greater in a microcentrifuge) for two minutes to separate the phases and transfer the aqueous phase to a suitably sized microcentrifuge tube.
10. Add 0.25 volume of 10 M ammonium acetate and 2.5 volumes of 100% ethanol to the sample and mix well.
11. Incubate at room temperature for 20 minutes, centrifuge to form pellet, wash the pellet several times with cold 70% ethanol, air-dry the pellet, and resuspend in a suitable buffer.

## References

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JWM 02/05

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