Locking PLGEL Heavy

Cat. no. : TGPLGH、TGPLGH-2

Storage: Store at room temperature, stable for at least 1 year.

Product Size: 200 tubes

Cat. no.	Product	Size
TGPLGH	Locking PLGEL Heavy 1.5 ml	200 tubes
TGPLGH-2	Locking PLGEL Heavy 2 ml	200 tubes

Introduction

Locking PLGEL (PLG) provides increased protection and ease of handling when working with standard organic extraction mixtures. The use of PLG can result in the recovery of 20% to 30% more nucleic acid compared with the use of traditional methods.

After organic extraction, it is often difficult to recover nucleic acid in the aqueous upper phase, which is free from the denatured protein present at the aqueous and organic phase interface. PLG present during phenol, phenol:chloroform, and chloroform:isoamyl alcohol extractions migrates under centrifugal force to form a seal between the organic and aqueous phases. The organic phase and interface material are effectively trapped below the PLG. 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.

PLG is inert, heat-stable, and does not interfere with standard nucleic acid restriction and modification enzymes. In fact, many of the reactions can be conducted in the presence of PLG at the appropriate temperature and then terminated by extraction with phenol or phenol:chloroform. PLG can be present during the heat inactivation of enzymes (65°C for 10 minutes) prior to the organic extraction.

The ability of PLG to separate the phases is based on the density differences of the aqueous and organic media. The organic layer must have a higher density than the PLG and aqueous phase, and the PLG must have a higher density than the aqueous phase.

Materials supplied with the kits

Various tube sizes are available with predispensed PLG Heavy. Sample volume ranges are indicated for each tube size.

PLG tube size	Sample volume	Tube color
1.5 ml, Heavy	100-500 µl	yellow
2 ml, Heavy	10-750 µl	yellow

The maximal centrifugal force for 15 mL and 50 mL conical tubes is 3,500 × *g*.

Applications and compatibility

a. Samples may be performed with PLG Heavy in combination with PCI, PC, or CI. However, PLG Heavy is not compatible with water- or Tris-buffer saturated Phenol (pH 8.0).

PCI = phenol:chloroform:isoamyl alcohol (25:24:1)

PC = phenol:chloroform (1:1)

CI = chloroform:isoamyl alcohol (24:1)

b. Bacterial cleared lysates are prepared according to the alkaline lysis procedure.

c. Total RNA is prepared using guanidinium isothiocyanate in conjunction with organic extraction.

PLG is not recommended for use with RNAzol®. In most cases, PLG Heavy can be used in combination with TRIzol® or RNA-Isol Lysis Reagent for Total RNA isolation. Use the volumes recommended in the TRIzol® or RNA-Isol Lysis Reagent protocol and the centrifuge speeds and times in the PLG protocol. In some special applications, it may be necessary to add to another volume of chloroform to achieve optimal phase separation.

PLG Heavy facilitates most applications that require extraction with organic solvents.

PLG Heavy may be used to prepare plasmid DNA from *E. coli* for the preparation of total RNA through homogenization in guanidinium isothiocyanate followed by organic extraction as well as for isolating genomic DNA from mouse tails.

Protocol

If not otherwise stated, all centrifugation is performed at room temperature, and expressed at r_{max}.

Caution: Be certain to use the correct centrifuge tube adapters to ensure tubes are properly supported on the sides and bottom.

Tube size	Sample volume
1.5 ml	100 - 500 µl
2 ml	100 - 750 µl

1. Immediately prior to use, pellet PLG at 12,000–16,000 × g in a microcentrifuge for 20–30 seconds.
2. Add 100 to 500 µL (PLG 1.5 mL), or 100 to 750 µL (PLG 2 mL) of aqueous sample and organic extraction solvent mix (e.g., if 0.2 mL of chloroform is added per 1 mL of TOOLSmart RNA

Extractor [cat. No. DPT-BD24] or TRIzol® Reagent used for homogenization, the mix will be 1,200 µL) directly to the prespun PLG tube.

3. Thoroughly mix the organic and aqueous phases to form a transiently homogeneous suspension. Do not vortex.
4. Centrifuge at 12,000–16,000 × g for 5 minutes to separate the phases. The PLG will form a barrier between the aqueous and organic phases. A small amount of PLG may remain in the bottom of the tube. If a second extraction is necessary and the maximum tube volume is not exceeded, more organic extraction solvent can be added to the same tube, mixed, and recentrifuged.
5. Carefully decant or pipette the nucleic-acid-containing aqueous upper phase to a fresh tube.
6. Precipitate the nucleic acid by adding salt, alcohol, and carrier (if required).

Applications

Mouse tail genomic DNA isolation protocol

Note: Genomic DNA is fragile. High-molecular-weight DNA is sheared easily by mechanical forces. Use suitable large-bore pipette tips or equipment when pipetting genomic DNA. Do not vortex solutions containing genomic DNA.

1. Place a 1-cm tail sample into a 1.5-mL microcentrifuge tube; this may be stored at −20°C. To minimize possible cross-contamination, do not mince the sample. Add 700 µL of lysis buffer (50 mM Tris-HCl, pH 8.0, 100 mM EDTA, 100 mM NaCl, 1% SDS) to the sample.
2. Add 35 µL of 10 mg/mL Proteinase K to the sample and mix briefly.
3. Incubate at 55 to 60°C overnight with mixing. This step should result in the complete solubilization of the tail fragment. In the case of incomplete digestion, more Proteinase K can be added and the samples incubated for several more hours.
4. Add 20 µL of 10 mg/mL RNase A (DNase-free) to the sample. Mix briefly and incubate at 37°C for 1–2 hours.
5. Transfer the entire solution to a prespun (1,500 × *g* for 1–2 minutes) PLG 2-mL Heavy tube.
6. Add 0.5 mL of phenol:chloroform:isoamyl alcohol (PCI, 25:24:1) to the sample in the PLG 2-mL tube and mix well by repeated inversion. Do not vortex.
7. Centrifuge at full speed (12,000 × *g* or greater) for 5 minutes in a microcentrifuge, and then carefully transfer the resultant aqueous phase to a fresh prespun PLG 2-mL Heavy tube.
8. Add 0.5 mL of chloroform:isoamyl alcohol (CI, 24:1) to the sample in the PLG 2-mL tube and mix well through repeated inversion. Do not vortex.

9. Centrifuge at full speed (12,000 × *g* or greater) for 5 minutes in a microcentrifuge, and then carefully transfer the resultant aqueous phase to a fresh microcentrifuge tube.
10. Fill the sample-containing tube with 100% isopropanol and mix thoroughly by repeated inversion. Do not vortex. A visible DNA precipitate should form. Proceed immediately to step 11.
11. Recover the DNA precipitate by touching it against a heat-sealed glass micropipette tip or by lifting the DNA with a yellow pipette tip with partial suction from a pipettor. Transfer the DNA to a 1.5-mL microcentrifuge tube containing 70% ethanol. If the DNA is not stringy, pellet through brief, low-speed centrifugation.
12. Wash the DNA with 70% ethanol, and then wash twice with 95% ethanol.
13. Allow the DNA to partially dry and then either transfer it to a microcentrifuge tube containing 400 µL of TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0) or add 400 µL of TE to the DNA in the tube. Do not vortex or repipette to resuspend DNA.
14. Resolubilize the DNA overnight (e.g., by rotation at 30–60 rpm). Resolubilization may be facilitated by heating the sample to 50°C.

Total RNA isolation protocol

1. Sample preparation:

   To extract RNA from washed and pelleted cultured cells, add 200 µL of 4 M guanidinium isothiocyanate solution (4 M guanidinium isothiocyanate, 25 mM sodium citrate, pH 7.0, 0.1 M ß-Mercaptoethanol) to 0.5×10^4 cells − 1×10^6 cells.

   To directly extract RNA from cultured cells growing in the monolayer, add 200 µL of 4 M guanidinium isothiocyanate solution directly to each well of a 6-, 12-, or 24-well plate. Add 100 µL of the 4 M guanidinium isothiocyanate solution directly to each well of a 48- or 96-well plate.
2. Homogenize monolayer cells by pipetting the mixture up and down several times, taking care to "wash" cell material free from the culture dish, tube, or well in the process. Homogenize washed, pelleted cells by pipetting the mixture up and down until the pellet is fully suspended. Use a small-bore pipette tip to collect the cell homogenate.
3. Transfer all the homogenate to a prespun (12,000–16,000 × g for 1–2 minutes) PLG 2-mL Heavy tube.
4. Add 20 µL (10 µL per sample for 48- or 96-well plates) of 2.0 M sodium acetate pH 4.0 to the sample, cap the PLG tube, and mix briefly.
5. Add 200 µL (100 µL per sample for 48- or 96-well plates) of water-saturated phenol to the sample,

cap the PLG tube, and mix thoroughly by repeated inversion. Do not vortex.

6. Add 60 µL (30 µL per sample for 48- or 96-well plates) of chloroform:isoamyl alcohol (CI, 49:1) to the sample in the same PLG tube and mix thoroughly by repeated gentle inversion. Do not vortex.
7. Incubate on ice for 10 minutes.
8. Centrifuge at 12,000–16,000 × g for 5 minutes in a microcentrifuge to separate the phases.
9. Add 200 µL (100 µL per sample from 48- or 96-well plates) of phenol:chloroform:isoamyl alcohol (PCI, 50:49:1) to the aqueous phase in the same PLG tube. Mix thoroughly by repeated gentle inversion. Do not vortex.
10. Centrifuge at 12,000–16,000 × g for 5 minutes to separate the phases.
11. Collect the resultant aqueous phase in an RNase-free microcentrifuge tube, add an equal volume of 100% isopropanol, and mix by repeated inversion.
12. Centrifuge at 12,000–16,000 × g for 20 minutes.
13. Discard the resultant supernatant and wash pellet several times with 200 µL of 70% ethanol, centrifuging for 2–3 minutes at 12,000–16,000 × g to repellet the sample.

Note: Samples may be stored in the 70% ethanol wash at this stage at −70°C or colder for extended periods.

14. Discard the final wash and dry the pellet at room temperature.
15. Redissolve the pellet in a suitable volume (5 to 10 µL) of RNase-free water. Store the RNA solution at −70°C.

Note: Absorbance determinations should be performed in RNase-free TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0).

Troubleshooting

Problem	Possible Cause	Resolution
PLG is not phasing properly	・The formulation may not be correct. ・The centrifuge speed may be incorrect. ・The PLG may have been frozen. ・The PLG was not spun down to the bottom of the tube prior to the extraction. ・The formulation may not be correct.	・Check the compatibility chart in Section 7 for the correct formulation of PLG. Check the protocol to ensure the centrifuge speed is correct. ・Store the PLG at room temperature. Spin the PLG to the bottom of the tube before performing the extraction. ・Check the compatibility chart in Section 7 for the correct formulation of PLG.
The PLG migrates to the top of both the aqueous and organic phases.	・The aqueous layer is denser than the PLG. ・The formulation may not be correct.	・Remove the liquid under the PLG by piercing the PLG with a pipette tip. ・Use a second pipette tip to recover the liquid and transfer to another prespun PLG tube. Add molecular biology grade water or an appropriate buffer to dilute the sample. ・Continue as described in the protocol. ・Check the compatibility chart in Section 7 for the correct formulation of PLG.
PLG remains on the bottom of the tube.	・The organic phase is not dense enough to remain below the	・Add chloroform to increase the density of the organic phase.

PLG is phasing but does not appear uniform.		・If the barrier is intact, proceed with the protocol. ・If there appears to be a hole or space in the barrier, retrieve the sample and place in a new PLG tube.