

Nucleic Acid Purification

Genomic DNA Purification

GenElute™ Plant Genomic DNA Miniprep Kits

The GenElute™ Plant Genomic DNA Miniprep Kit permits purification of high quality genomic DNA from a variety of plant species (Table 1, Fig. 1 and 2). The kit contains all the reagents, columns and tubes necessary to isolate genomic DNA from up to 100 mg of fresh or 20 mg of freeze-dried plant tissue. Plant tissue is disrupted by grinding in liquid nitrogen, and DNA is released with detergent and chaotrope. Proteins, polysaccharides, and cell debris are eliminated with a 10 minute precipitation procedure followed by centrifugation through a filtration column, included in the kit. The genomic DNA is purified further by a silica bind-wash-elute procedure in microcentrifuge spin columns. Purified DNA is ready for downstream applications such as PCR (Fig. 3), restriction endonuclease digestion, cloning and Southern blots.

Features and Benefits

- Less than 40 minutes from tissue to purified genomic DNA, including disruption in liquid nitrogen (Fig. 4)
- Typical DNA yields of up to 20 µg per prep
- No RNase treatment required
- 40% more purifications per kit than leading supplier

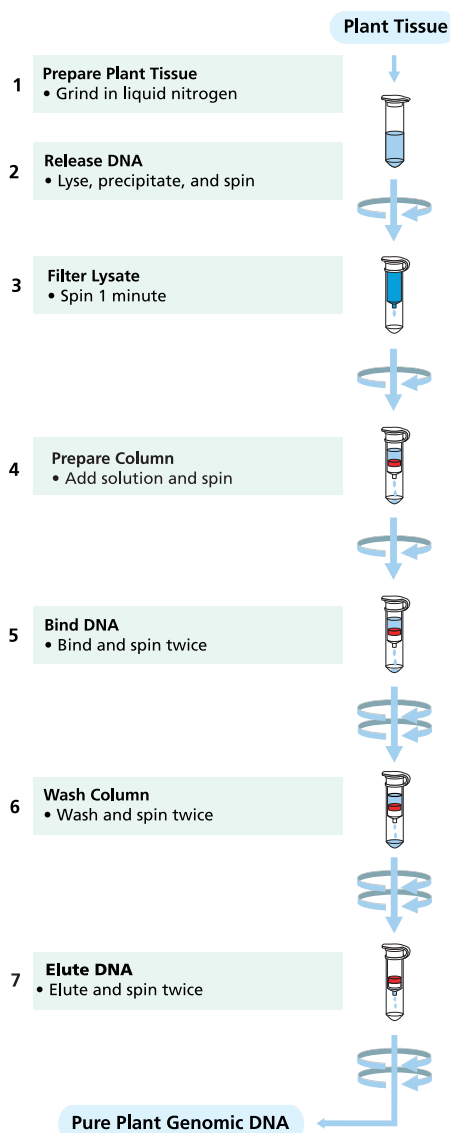
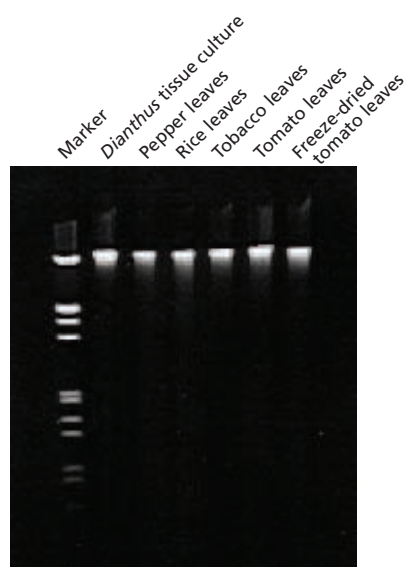


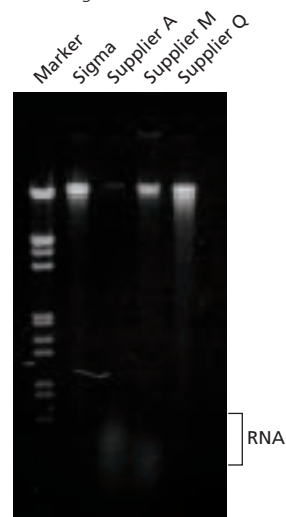
Table 1. Typical yields of genomic DNA isolated from various plant species per 100 mg of starting leaf tissue.

Material	Typical Yield
Corn	7.5 µg
Dianthus tissue culture	3.3 µg
Pepper	3.1 µg
Rice	5.9 µg
Soybean	5.7 µg
Tobacco	5.2 µg
Tomato	6.2 µg
Tomato (20 mg freeze dried leaf tissue)	5.7 µg
Wheat	11.5 µg



Genomic DNA from various plant species isolated with GenElute™ Plant Genomic DNA Miniprep Kit.

Figure 1. Purified genomic DNA (0.4 µg/lane) was analyzed on a 0.8% agarose gel. Marker is lambda *Hind* III digest.



Genomic DNA isolated from 100 mg fresh tomato leaves using various kits.

Figure 2. Purified genomic DNA (0.4 µg/lane) was analyzed on a 0.8% agarose gel. Sigma, membrane-based; Supplier A, resin-based; Supplier M, solution based; Supplier Q, membrane-based. Marker is lambda *Hind* III digest.

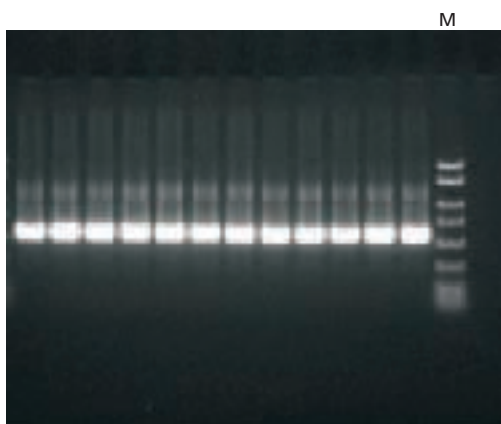
Note: RNA contamination is present in DNA isolated using the kits from both Supplier A and M.

Nucleic Acid Purification

Genomic DNA Purification

(Continuation of)

GenElute™ Plant Genomic DNA Miniprep Kits



PCR amplification of a 500 bp product isolated from genomic DNA.

Figure 3. Genomic DNA from soybean leaves was purified using the GenElute™ Plant Genomic DNA Miniprep Kit. A 5 µl aliquot of eluate was used as template in a 20 µl total PCR reaction for 30 cycles. A 5 µl aliquot of each PCR reaction was resolved on a 2% precast agarose gel (Product Code 5722). The PCR marker (M) used (Product Code P 9577) ranged from 50 bp to 2 kb.

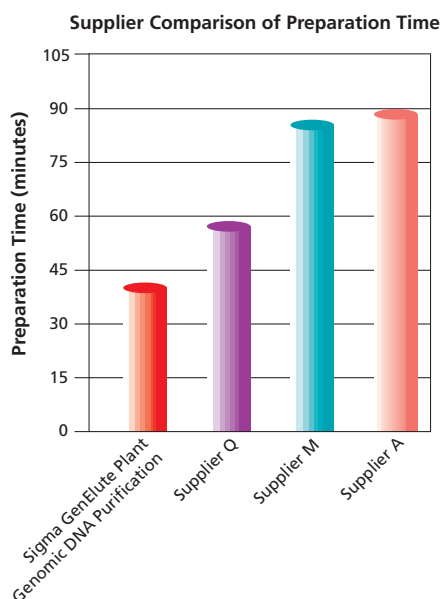


Figure 4. Comparison of preparation time. The prep time required using the GenElute™ Plant Genomic DNA Miniprep Kit, compared to kits from three other suppliers.

G2N-10 GenElute™ Plant Genomic DNA Miniprep 1 kit

Kit
 sufficient for 10 purifications

R: 20/21/22-32-36/38 S: 26-36

G2N-70 GenElute™ Plant Genomic DNA Miniprep 1 kit

Kit
 sufficient for 70 purifications

G2N-350 GenElute™ Plant Genomic DNA 1 kit

Miniprep Kit
 sufficient for 350 purifications

Plasmid DNA Purification

GenElute™ Plasmid Miniprep Kits

The GenElute Plasmid Purification Kits offer simple, rapid, and cost-effective methods for isolating plasmid DNA from *E. coli* cultures. These kits combine silica-based membrane technology and the convenience of a spin column format. These kits also recover up to 20 µg of high copy plasmid DNA per ml of overnight culture.

Bacterial cells are harvested via centrifugation, subjected to a modified alkaline-SDS lysis procedure and the DNA adsorbed onto silica in the presence of high salts. Contaminants are then removed by a simple wash step.

Finally, the bound DNA is eluted in water or Tris-EDTA buffer. The recovered plasmid DNA is predominately in its supercoiled form. There is no visual evidence of genomic DNA or RNA contamination. The DNA is ready for immediate use in applications such as restriction enzyme digestion, cloning, PCR, transformation, transcription, conventional, and automated sequencing (Fig. 1).

Features and Benefits

- 40% more preps per kit than the leading supplier
- Purify up to 20 µg of plasmid DNA per ml of culture
- Purified plasmid DNA in less than 30 minutes for up to 24 preps
- Faster than gravity flow anion exchange columns
- No detectable genomic DNA or RNA contamination
- No phenol/chloroform extraction or alcohol precipitation required
- Contains additional wash buffer for use with EndA⁺ *E. coli* bacterial strains (e.g., HB101, JM101, BL21)

R: 10-21/22-36/37/38 S: 16-26-36/37

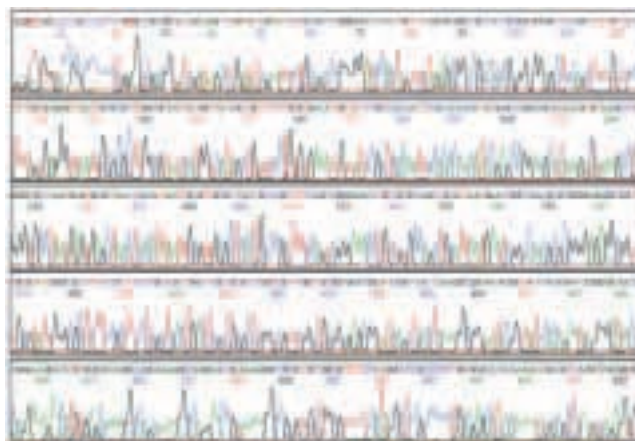
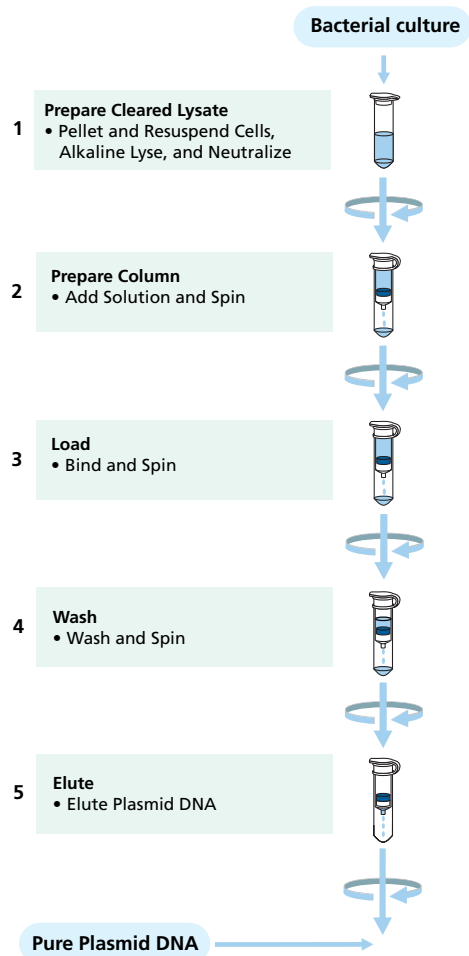


Figure 1. Electropherogram revealing >600 bases of sequence from pUC-TMV using GenElute Plasmid DNA Purification Miniprep Kit. Cycle Sequencing was performed using 500 ng of template, a T7 sequencing primer, and ABI BigDye™ terminator chemistry. Sequencing reactions were resolved on an ABI Prism 377 XL instrument with a 48 cm gel cassette containing 4.5% AutoPAGE Plus acrylamide at 2.4 kV, 48 °C for 7 hours.

Nucleic Acid Purification

Plasmid DNA Purification



PLN-10 GenElute™ Plasmid Miniprep Kit sufficient for 10 purifications 1 kit

RT



PLN-70 GenElute™ Plasmid Miniprep Kit sufficient for 70 purifications 1 kit

RT



PLN-350 GenElute™ Plasmid Miniprep Kit sufficient for 350 purifications 1 kit

RT

GenElute™ High Performance (HP) Plasmid Kits

RT

NEW

The GenElute™ High Performance (HP) Midiprep and Maxiprep Kits offer purification of high quality plasmid DNA in less than 30 minutes (Fig. 1). GenElute HP Plasmid kits feature a filter syringe for lysate clearing, and binding columns that can be used with a vacuum or spin column format.

An overnight recombinant *E. coli* culture is harvested by centrifugation and subjected to a modified alkaline-SDS lysis procedure. The lysate is cleared with a filter syringe and DNA is bound to the silica-based membrane. The remaining contaminants are removed by a wash step. Finally, the bound DNA is eluted in buffer or water (Fig. 2). This high quality DNA is suitable for the most demanding applications including transfection (Fig. 3), restriction digestion (Fig. 4), ligation, automated sequencing (Fig. 5).

Features and Benefits

- From harvested bacterial culture to pure plasmid DNA in 30 minutes or less
- Up to 350 µg (Midi) and 1.2 mg (Maxi) yield of high-copy plasmid DNA
- Offers the flexibility of a vacuum or spin format
- Contains fewer plastic components than other HighSpeed Kits, reducing the amount of waste
- No phenol/chloroform extraction or alcohol precipitation required

R: 22-36/37/38 S: 26-36



Solutions are drawn through the column by vacuum (see above) instead of centrifugation, increasing speed and decreasing hands on time.



GenElute High Performance (HP) Plasmid Kits

Nucleic Acid Purification

Plasmid DNA Purification

(Continuation of)

GenElute™ High Performance (HP) Plasmid Kits

Preparation Time for different Maxiprep plasmid purification kits

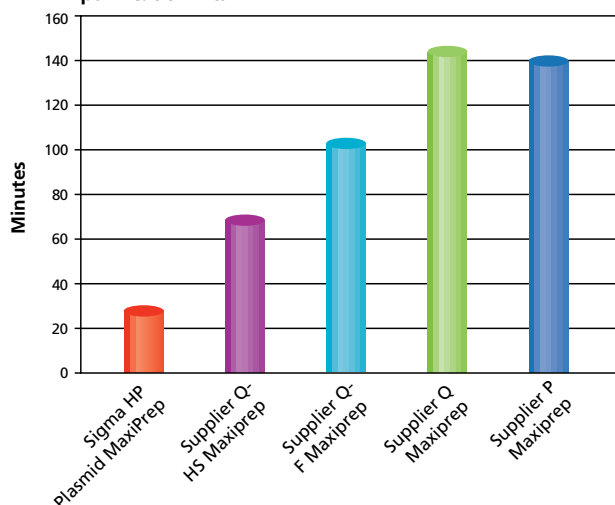


Figure 1. Comparison of time required per isolation using different Maxiprep plasmid isolation systems. Plasmid DNA was isolated following each manufacturer's recommended protocol. Each system was tested multiple times and the average preparation time is shown here.

Plasmid DNA Yield for different Maxiprep Kits

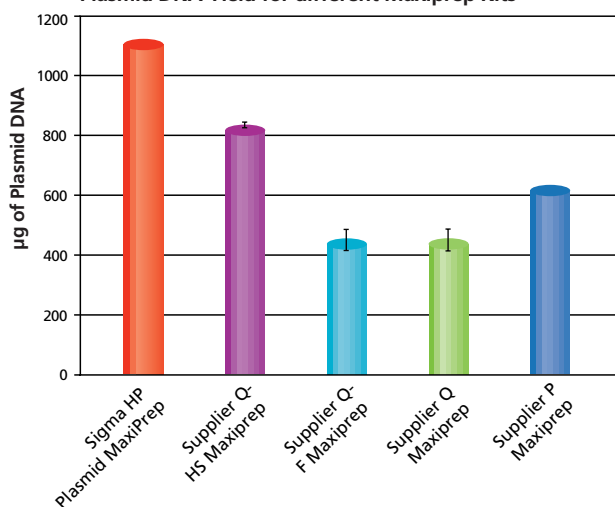
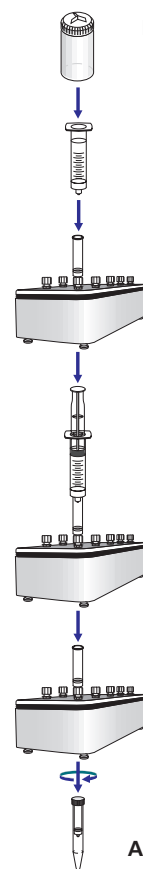


Figure 2. Comparison of plasmid DNA yield using different Maxiprep plasmid isolation systems. Plasmid DNA was isolated following each manufacturer's recommended protocol. All samples were done in duplicate.



HP - Midi or MaxiPrep

- 1.) Resuspend and Lyse cells – 3-5 minutes
- 2.) Neutralize in filter syringe – 5 minutes
- 3.) Prepare column
 - Add solution and vacuum
- 4.) Clear lysate and bind DNA
 - Filter lysate directly onto column and vacuum – 5 minutes
- 5.) Wash
 - Add wash solution and allow column to dry on vacuum – 10 minutes
- 6.) Elute
 - Centrifugation – 5 minutes

Purified Plasmid DNA
Actual Time: Under 30 minutes

Supplier Q-HS MaxiPrep

-
- 1.) Resuspend and Lyse cells – 5 minutes
 - 2.) Neutralize in filter syringe – 10 minutes
 - 3.) Prepare column
 - Add solution and allow to drip
 - 4.) Clear lysate and bind DNA
 - Filter lysate directly onto column and drip
 - 5.) Wash
 - Add wash solution and allow to drip
 - 6.) Elute
 - Add Elution and allow to drip
 - 7.) Precipitate DNA
 - Add Isopropanol incubate
 - 8.) Prepare precipitation device
 - 9.) Bind DNA
 - Filter eluate through device
 - 10.) Wash
 - Add 70% ethanol
 - 11.) Dry precipitation device
 - 12.) Elute DNA
 - Elute DNA off device twice.

Purified Plasmid DNA
Actual Time: 60 minutes

Nucleic Acid Purification

Plasmid DNA Purification

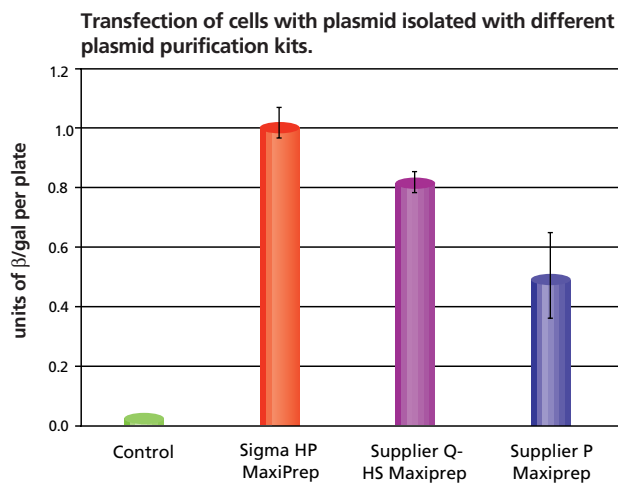
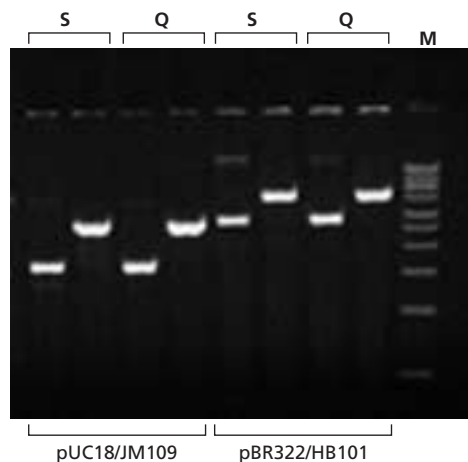


Figure 3. Comparison of transfection efficiencies of plasmids prepared with different plasmid isolation methods. Data shows an average of 3 replicates for each named isolation method. All transfections were in CHO-K1 cells. The OD measurements were taken at 420 nm recording the units of β-gal/plate. The cells were grown to 60-70% confluency and transfected using 3 μg of plasmid pSPORT-CMV/15 μg of Escort IV. β-Gal activity was determined 54 hours post-transfection.



Purified plasmid DNA is suitable for restriction enzyme digestions.

Figure 4. Restriction digestion of plasmid DNA isolated using the GenElute™ HP Plasmid Maxiprep Kit and a comparable kit from Supplier Q. The plasmid DNA (800 ng) was digested with 10 units of Hind III at 37 °C for 4 hours. Each plasmid sample represents 100 ng of undigested DNA (left) and digested DNA (right) loaded in each pair of lanes. Samples were loaded on a 1% TBE agarose gel. The marker (M) used was a 1 kb DNA Ladder (Product Code D 0428).

High Quality DNA for Automated Sequencing

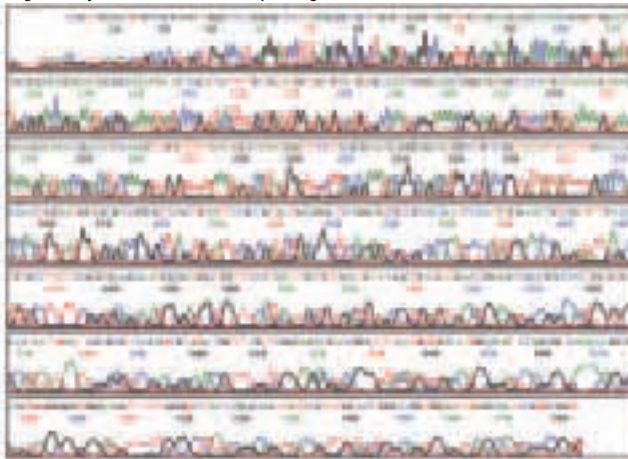


Figure 5. Electropherogram revealing >700 bases of sequence from pCMV-SPORT-βgal purified from GenElute™ HP Plasmid Midiprep Kit. Cycle Sequencing was performed using 500 ng template, a T7 sequencing primer and ABI BigDye™ terminator chemistry. Sequencing reactions were resolved on an ABI Prism® 377 XL instrument with a 48 cm gel cassette containing 4.5% AutoPAGE Plus acrylamide at 2.4 kV, 48 °C for 7 hours.

NA0200S RT	GenElute™ HP Plasmid Midiprep Kit	1 kit
	sufficient for 4 purifications	
NA0200 RT	GenElute™ HP Plasmid Midiprep Kit	1 kit
	sufficient for 25 purifications	
NA0300S RT	GenElute™ HP Plasmid Maxiprep Kit	1 kit
	sufficient for 4 purifications	
NA0300 RT	GenElute™ HP Plasmid Maxiprep Kit	1 kit
	sufficient for 10 purifications	
NA0310 RT	GenElute™ HP Plasmid Maxiprep Kit	1 kit
	sufficient for 25 purifications	

PhasePrep™ BAC DNA Kit

NA0100
RT

The PhasePrep BAC DNA Kit offers a highly scalable, rapid, cost-effective method for isolating high molecular weight plasmids such as Bacterial Artificial Chromosomes (BACs) from recombinant *E. coli* cultures.

The kit utilizes a phase separation procedure that can be scaled to micro, mini, midi, or maxi prep sizes. Sufficient reagents are provided for 300 micro, 180 mini, 30 midi, or 15 maxi preps. Up to 2, 12, 60, or 100 μg of BAC DNA can be recovered from 5, 40, 250, or 500 ml of overnight recombinant *E. coli* culture, respectively. The purified BAC DNA contains very low levels of endotoxins and is ready for immediate use in automated sequencing (Fig. 1), PCR, restriction enzyme digestion (Fig. 2), cloning and other common applications.

Features and Benefits

- Typical DNA yields of 2-100 μg from 5-500 ml of overnight cultures
- No phenol/chloroform extraction required
- Allows possible Micro to Maxi preps with the same kit
- No long waits for drip columns

R: 11-25-34-67 S: 16-26-37/39-45

Nucleic Acid Purification

Plasmid DNA Purification

(Continuation of)

PhasePrep™ BAC DNA Kit

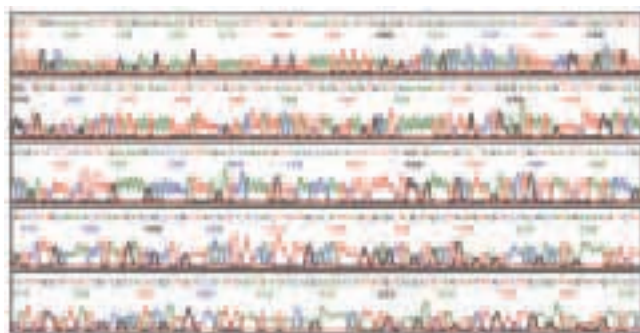
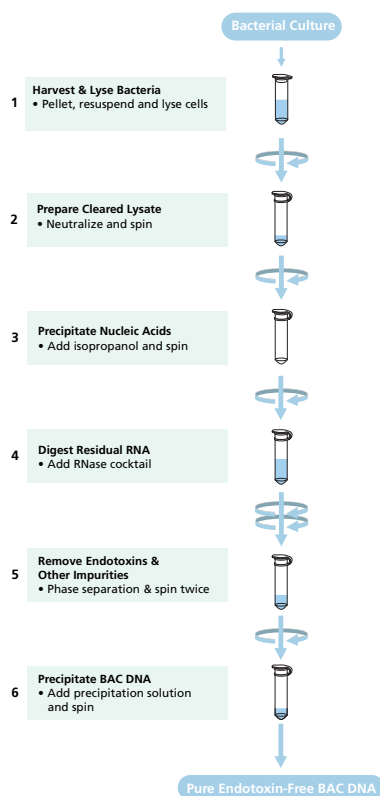
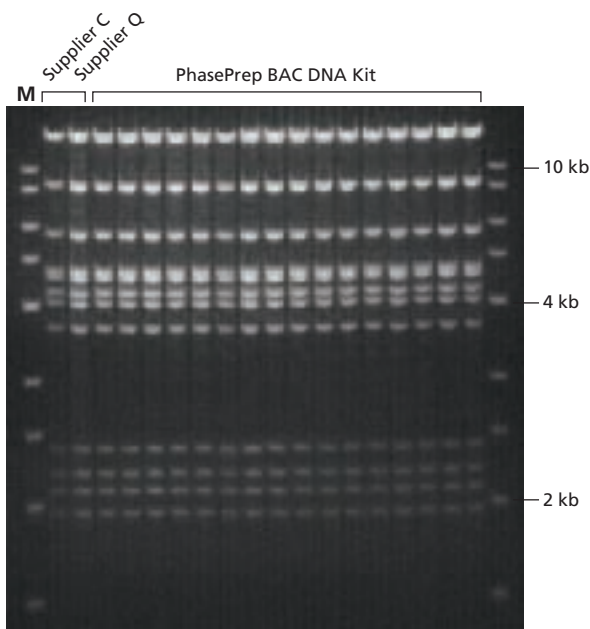


Figure 1. BAC DNA was purified with the PhasePrep™ BAC DNA Kit and sequenced with a custom primer using BigDye™ Terminator Chemistry. Sequencing reactions were resolved on an ABI 3700. Data provided by the Genome Sequencing Center at Washington University, St. Louis



Purified BAC DNA is suitable for restriction digestion.

Figure 2. Restriction digestion of BAC DNA isolated with the PhasePrep™ BAC DNA Kit and two other suppliers (C and Q). BAC DNA samples were purified from overnight cultures of *E. coli* HB101b transformed with a pbelloBAC11 clone. Approximately 1 µg of DNA from each sample was digested with 20 units of EcoR V at 37 °C for 4 hr. One half of each digestion (approximately 0.5 µg) was separated by overnight electrophoresis in 1% agarose gel. Lanes 1-4 were BAC DNA purified with Sigma kit, lane 1 was BAC DNA purified with supplier C's kit, and lane 2 was BAC DNA purified with Supplier Q's kit. The molecular marker used at the first and last lanes was a 1 kb DNA Ladder (Product Code D 0428).

RNA Purification

TRI® Reagent

T 9424 TRI® Reagent is an improved version of the 25 mL single-step total RNA isolation reagent 100 mL developed by Chomczynski.¹ The RNA 200 mL isolation method based on this reagent

is widely used and proven for RNA applications². It is ideal for quick, economical, and efficient isolation of total RNA or the simultaneous isolation of RNA, DNA, and proteins from samples of human, animal, plant, yeast, bacterial, and viral origin. For processing tissues, cells cultured in monolayer, or cell pellets.

Features and Benefits

- Quick and convenient reagent for use in the simultaneous isolation of RNA, DNA and protein.
- Performs well with large or small amounts of tissue or cells and many samples can be simultaneously extracted.
- One of the most effective methods for isolating total RNA. Purifications can be completed in only 1 hour starting with fresh tissue or cells.

1 ml sufficient for 10⁷ cells

1 ml sufficient for 100 mg tissue

References

1. Chomczynski, P. and Mackey, K., Modification of the Tri Reagent procedure for isolation of RNA from polysaccharide- and proteoglycan-rich sources. *Biotechniques* **19**, 924 (1995)
2. Chomczynski, P. and Sacchi, N., Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**, 156 (1987)

R: 24/25-31-34 S: 26-27-36/37/39-45

Nucleic Acid Purification

RNA Purification

RNAlater™

R 0901 RNAlater is an aqueous, non-toxic tissue and cell storage reagent that stabilizes and protects cellular RNA in intact, unfrozen tissue and cell samples. RNAlater eliminates the need to immediately process samples or to freeze samples in liquid nitrogen for later processing. RNAlater can be used with various downstream applications including mRNA and total RNA isolation, histology and immunocytochemistry and is compatible with Sigma's GenElute isolation kits.

RNAlater is easy to use. Simply cut tissue samples to be stored so they are less than 0.5 cm in at least one dimension and submerge in 5 volumes of RNAlater. When ready to isolate the RNA, remove the tissue from RNAlater and process as though just harvested. For cell storage, resuspend pelleted cells in a small amount of PBS before adding 5-10 volumes of RNAlater. Before preparing RNA, pellet cells and discard supernatant.

Features and Benefits

- Rapidly permeates tissues to stabilize and protect cellular RNA with immediate RNase inactivation
- Stabilizes samples at room temperature for up to one week or indefinitely at -20 °C for archiving needs
- No compromise in RNA quality following mRNA or total RNA isolation
- Aqueous non-toxic solution allows downstream tissue processing

Licensed for distribution by Ambion, Inc., The RNA Company. RNAlater is a registered trademark of Ambion, Inc., Austin, Texas and is protected by various U.S. and foreign patents.

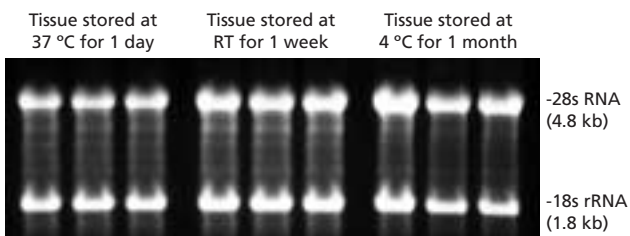
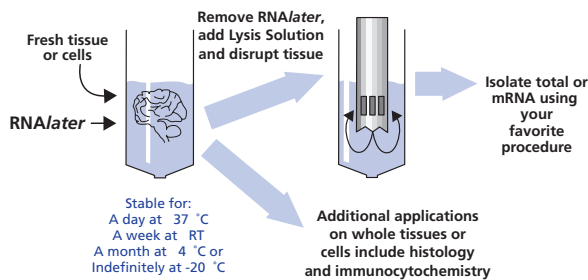
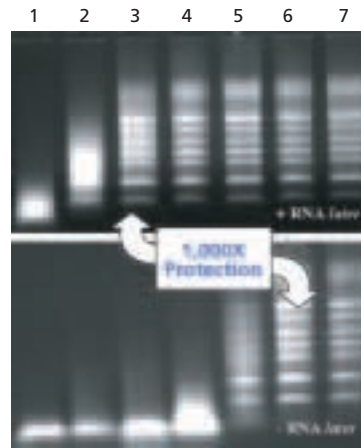


Photo courtesy of Ambion.

Stabilizes Samples at Room Temperature For Up To One Week

Quality of RNA isolated from tissue stored in RNAlater solution. Fresh tissues were stored in RNAlater at 37 °C for 1 day, room temperature for 1 week, or 4 °C for 1 month. RNA was isolated using TRI[®] Reagent (Product Code [T 9424](#)) and analyzed using denaturing agarose gel electrophoresis.



Superior Protection Against RNase Degradation

A 5 ml aliquot of RNase A (Product Code [R 6513](#); serially diluted to final concentrations of 4.5×10^{-5} – 4.5×10^{-11} units/ μ l) was added to 5 mg RNA (Product Code [R 7020](#)) in 15 μ l containing either 10 μ l of RNAlater (*top panel*) or TE buffer (*bottom panel*). Reactions were incubated at 37 °C for 20 minutes, purified using the GenElute™ Total RNA kit (Product Code [RTN10](#)) and analyzed on a 1% agarose gel.

DNase I

AMP-D1

-20°C



WET ICE

(Deoxyribonuclease I)

1 kit

EC 3.1.21.1

Amplification Grade

Because PCR can detect even a single molecule of DNA, RNA samples should be digested with DNase I before RT-PCR, and parallel reactions should be run without reverse transcriptase to check for amplification of contaminating DNA. DNase I digests double- and single-stranded DNA into oligo- and mononucleotides. Using the Reaction Buffer provided, DNA is removed from RNA preparations in a 15 minute digestion at room temperature. The DNase I is then inactivated by heating with the Stop Solution. Heating also denatures hairpins in the RNA, so the RNA can be used directly in reverse transcription.

Many commercial DNase I formulations are contaminated with residual RNases. This RNase contamination can destroy or degrade valuable RNA samples prior to reverse transcription. Laboratory comparisons have shown that Sigma's Amplification Grade DNase I demonstrates lower RNase activity than that from several leading molecular biology product suppliers (Fig. 1).

Features and Benefits

- Ideal for eliminating DNA from RNA preparations prior to sensitive applications such as RT-PCR
- Lowest RNase activity available
- Includes optimized 10x reaction buffer and Stop Solution

Unit definition: One unit completely digests 1 μ g of plasmid DNA to oligonucleotides in 10 min. at 37 °C.

Components:

DNase I, 1,000 units

10X Reaction buffer, 1 mL

Stop solution, 1 mL

The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

Nucleic Acid Purification

RNA Purification

(Continuation of)
DNase I

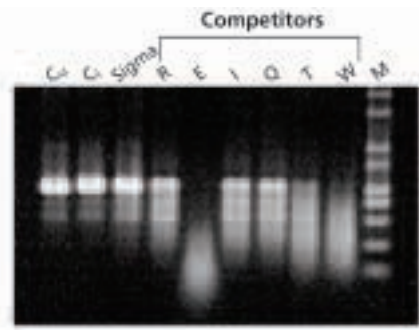


Figure 1: Sigma Amplification Grade DNase I has the lowest RNase activity.

For Sigma DNase I, and for each competitor's DNase I, the following assay was completed: 1 µg of a 1.9 kb *in vitro* transcription product was incubated with 1 unit of the respective DNase I at 37 °C for 1 hour and analyzed on a 1% agarose gel.

C_u = unincubated control (RNA in buffer without DNase, kept on ice).

C_i = incubated control (RNA in buffer without DNase, incubated at 37 °C for 1 hour).

Note: To determine the effectiveness of DNase I treatment, parallel PCR reactions should be run without adding reverse transcriptase to check for amplification from contaminating DNA.

Ribonuclease Inhibitor

R 2520 Inhibits RNase by forming in a tight, 2500 units
non-covalent 1:1 complex. 10000 units
This inhibitor of RNase activity is 20000 units
isolated from human placenta and
supplied as a buffered aqueous glycerol solution.
Useful for *in vitro* inhibition of ribonucleases, including
procedures like cDNA synthesis, RT-PCR, and *in vitro*
transcription and translation.
Solution in 50% glycerol, 20 mM HEPES-KOH, pH 7.6, 50 mM
KCl and 8 mM DTT
Unit definition: One unit will reduce the activity of 5 ng of
ribonuclease A by 50% in a cytidine 2':3'-cyclic
monophosphate system.
mol wt approx. 50 kDa
Concentration. 30,000-50,000 units/mL
References
1. Blackburn, P., *J. Biol. Chem.* **254**, 12484 (1979)
2. Blackburn, P., Ribonuclease inhibitor from human placenta:
interaction with derivatives of ribonuclease A *J. Biol. Chem.* **254**,
12488-12493 (1979)
S: 24/25

ProtectRNA™ RNase Inhibitor 500× Concentrate

R 7397 2 ml treats 1,000 ml of solution. 30 mL
R: 10-36/37/38 S: 16-26-36/37/39

RNaseZAP

R 2020 A cleaning agent for removing RNase 250 mL
from glassware, plastic surfaces, 6 × 250 mL
countertops, and pipettors. It is also
effective at eliminating RNase contamination from
microcentrifuge tubes without inhibiting subsequent enzymatic
reactions.
R: 10-36/37/38 S: 16-26-36/37/39

Nucleic Acid Amplification

Routine PCR Amplification

Taq DNA Polymerase

from *Thermus aquaticus*

CAS No. 9012-90-2

WET ICE EC 2.7.7.7

Taq DNA Polymerase is a specialized thermostable enzyme isolated from the thermophilic bacterium *Thermus aquaticus*. The recombinant form of this enzyme is expressed in *E. coli*. This 94 kDa protein shows no detectable levels of contaminating endonucleases or exonucleases by SDS-PAGE. It has both 5'→3' polymerase and exonuclease activity.

Taq DNA polymerase comes with the choice of an optimized 10× reaction buffer including MgCl₂ or a 10× reaction buffer without MgCl₂ plus a separate tube of MgCl₂ for titration. The latter option may be necessary to determine optimal conditions for amplification.

Unit definition: One unit will incorporate 10 nmol of total dNTPs into acid-precipitable DNA in 30 min at 74 °C.

recombinant, expressed in *Escherichia coli*
suitable for polymerase chain reaction (PCR) and automated
sequencing reactions

Concentration. 5 units/µL

This product is sold under license from Roche Molecular Systems, Inc. and Applied Biosystems and the sale and use of this product are expressly limited and governed by a limited license - the details of which appear in full on the inside back cover of this product guide.

References

- Innis, M.A. et al., *PCR Protocols: A Guide to Methods and Applications* (1990),
- Innis, M.A. et al., *Proc. Nat. Acad. Sci. USA* **85**, 9436 (1988)
R: 36/37/38 S: 26

D 1806 Taq DNA Polymerase with 10× 50 units
reaction buffer containing MgCl₂ 20 × 250 units
1500 units
10 × 1500 units
5000 units

D 4545 Taq DNA Polymerase with 10× 50 units
reaction buffer without MgCl₂. 250 units
Includes a separate tube of 25 mM 20 × 250 units
MgCl₂ 1500 units
5000 units
5000 units