RPM® 4G

Rapid Pure Mini-Monster Prep

Revision No. 2078-999-8E04W

Overview

* 1 Prep Processed in ~ 2 Hours
* No Phenol/Chloroform Extraction
* No Slow Gravity Columns or Dedicated Equipment
* Yield Up to 10 mg Pure High Copy Number Vector DNA Per Prep

Shipping & Storage

The RPM 4G is shipped and stored at room temperature. Upon arrival, store the RNase Mixx at 4°C.

Kit Components
2078-200 (12 preps)

CAT. NO.	DESCRIPTION	SIZE/VOL.
2078-201	Cell Wash Concentrate	50 ml
2078-202	Alkaline Lysis Concentrate I	100 ml
2078-203	Alkaline Lysis Concentrate II	75 ml
2078-204	Neutralizing Solution	780 ml
2078-205	GLASSMILK® 4G	520 ml
2078-216	Wash Solution Concentrate**	15 ml
2078-207	RNase Mixx	6.2 ml
2078-208	5M NaCl	7.5 ml
2078-211	Bottle	1
2078-212	Filter Unit 4G	12
2078-213	DNA Trap Tube	12
2078-214	LiCl	120 ml
2078-215	Pre-Lysis Concentrate	75 ml
5080-201	Bottle Holder	1
5040-201	Sieve Material***	1

*The **GLASSMILK 4G** contains guanidine thiocyanate, use with proper precaution.
**Add 15 ml Wash Solution Concentrate (2078-216) to 300 ml distilled water and mix. Add 332 ml ETOH and mix well before first use.
***Sieve Material is reusable. Rinse well under running water and blot dry

User Supplied Wash Solution:

1:1 acetone/ethanol solutions: 25 ml acetone + 25 ml ethanol (store at room temperature).

Note: The **RPM 4G** Kit contains sufficient reagents and materials for 12 Mini-Monster preps.

Be Prepared: In order to efficiently utilize the kit, the following reagents and materials must be available and are **supplied by the user:** 95% ethanol, isopropanol, acetone, sterile water or TE buffer, 250 or 1000 ml centrifuge bottles and centrifuge; 50 ml centrifuge tubes and centrifuge rotor (preferably a swinging bucket model that will pellet plasmid precipitates on the bottom of the tubes); a funnel and a vacuum source.

Introduction

The **RPM 4G** kit is designed for rapid isolation and purification of double stranded plasmid DNA from (4 grams of) bacterial cells. It delivers high yields of supercoiled plasmid DNA with minimum effort and time. The resulting DNA is suitable for enzymatic manipulations including sequencing, restriction enzyme digestion, *in vitro* transcription and PCR. It is particularly useful for *in vitro* and *in vivo* transfection studies; **RPM 4G**-purified DNA gives comparable results to 2x CsCl purified DNA regardless of the transfection method employed (e.g., CaCl2 precipitation, Lipofectin, electroporation, injection) or the detection mode used (e.g., LacZ, CAT, luciferase).

The Kit accommodates one of three protocols that can be chosen based on specific needs. The differences between these protocols are described briefly and summarized in the table below. The **RPM 4G** Kit is designed to process 1 L LB/300 ml CIRCLEGROW cultures (High Copy Protocol). It can also process 3 L LB/1 L CIRCLEGROW cultures to enhance the yield from low copy number vectors (Low Copy Protocol). The Low Copy Protocol differs from the High Copy Protocol by the use of twice the volume of some reagents and the inclusion of an isopropanol precipitation to concentrate the plasmid DNA solution prior to matrix purification, simplifying the process of working with more cells.

The **RPM 4G** Kit is based on a modified alkaline lysis protocol followed by plasmid DNA purification on a special **GLASSMILK 4G Matrix**. In addition, vacuum filters are used with special membranes that eliminate impurities. They also make it possible to elute the DNA in a small volume allowing precipitation in a single tube. The resulting plasmid DNA is low in endotoxin and free of RNA, cellular DNA, and other contaminants for use in all types of studies including sensitive transfections. It is the only kit that specifically addresses elimination of contaminating cellular DNA and nucleases.

Elimination of Cellular RNA:

RNA is eliminated either enzymatically (RNases) or by selective precipitation with salts. Both of these treatments lower the RNA level sufficiently to prevent its co-purificaton with plasmid.

Elimination of Cellular DNA:
Bacterial genomic DNA, in the vast majority of cases, is eliminated by the modified alkaline lysis conditions employed. However, with certain host vector combinations, genomic DNA contamination is unavoidable; an optional step (in the Modified **High Copy** and **Low Copy** Protocols) is included that can be used to eliminate cellular DNA carry-over: DNA is heat denatured/renatured, and ssDNA is precipitated with high salt.

Elimination of Endogenous Endonucleases:
Certain bacterial host cells such as HB101 and RR1 are End A+ and contain higher levels of endogenous nuclease activity that can co-purify with the plasmid DNA. This is observed regardless of the matrix used: ion exchange or silica. The **GLASSMILK 4G Matrix** eliminates all nuclease activity in most cases; however, when processing large cell pellets, trace nuclease activity can be carried over sufficient to partially degrade the plasmid DNA in the presence of divalent cations. The **"Modified" High Copy** and **Low Copy** Protocols include an optional chloroform extraction for the purpose of eliminating trace nuclease activity in the final isolate.

Comparison of the High Copy, "Modified" High Copy and Low Copy Protocols

Features	**High Copy**	**"Modified" High Copy**	**Low Copy**
1 L LB/300 ml CIRCLEGROW	X	X	
3 L LB/1 L CIRCLEGROW		X	
Cell Wash	X	X	X
Pre-Lysis	25 ml	25 ml	50 ml
Lysis	25 ml	25 ml	50 ml
Neutralization	25 ml	25 ml	50 ml
Add RNase Mixx	X	Optional	Optional
Isopropanol precipitation		X	X
Prevent Cellular DNA carry-over**		X	X
Process bacteria with high endonuclease content**		X	X
GLASSMILK 4G	X	X	X
Equal to 2 x CsCl purified DNA		X	X
Equal to GENECLEAN pure DNA		X	X

** Optional Step.

High Copy Protocol		
Media 1 L {3} LB*	**300 ml {1L} CIRCLEGROW****	
Copy Number	High/Low	High/Low
Inoculation Source	2 ml culture***	0.6 ml culture***
Growth at 37°C	14-16 hours	up to 3 days
Yield(High Copy)1	3-10 mg	3-10 mg

Yield (Low Copy)2 0.2-1 mg 0.2-1 mg
For Low Copy Protocol, use the values in "{ }"
*LB Broth (see pg 216)
**CIRCLEGROW is a super-rich bacterial growth media (see pg 212)
***Overnight culture.
1 High copy vectors = pUC, pTZ, pGEM, pBS, etc. Typical yield with insert is 1-3 mg.
2 Low copy vectors = pBR 322, cosmids, etc. Typical yield with insert is 0.1-0.5 mg.
Note: High copy vectors can become low copy with certain inserts. The notation "high" copy can only be used as a rough guide when estimating plasmid yield from a given culture volume or cell pellet weight. Yields from plasmids containing inserts that differ by a single base can vary in yield by as much as 100 fold.

1. **Cell Lysis.**
Use values in { } when following Low Copy Protocol
a. Culture cells using the table above as a guideline. Pellet culture at 6000 x g for 10 min (e.g., 6000 rpm in GSA rotor with 250 ml buckets; 4000 rpm in HL-4 or TYJS5.2 rotor with 1 liter buckets) and discard media.

b. Resuspend cells in 40 ml H2O by vigorous shaking. Add 4 ml **Cell Wash Concentrate**; swirl to mix, and incubate 5 min at room temperature. Spin as in step a., decant supernatant and drain pellet. Cell Wash is important in reducing endotoxin levels in the final purified DNA.

c. Add 22.5 ml {45} dH2O and 2.5 ml {5} of **Pre-Lysis Concentrate**. Mix by vigorous shaking until the cells are completely resuspended (this is essential for efficient cell lysis and maximium DNA yield). Add 250 µl of **RNase Mixx** and invert to mix.

d. Prepare 25 ml {50} Alkaline Lysis Solution for each sample in a separate container: 2.5 ml {6.5} **Alkaline Lysis Concentrate I**, 2.5 ml {5} **Alkaline Lysis Concentrate II** and 20ml {38.5} H2O. (Note: Before using **Alkaline Lysis Concentrate II**, dissolve precipitated SDS by warming, if necessary). Add to cells and gently invert 15 times.

e. Add 25 ml {50} ice cold **Neutralizing Solution** and shake vigorously 3-5 times until a uniform white precipitate forms. Incubate 10 to 30 min on ice and spin at 6,000 x g for 10 min at 4°C. Collect the supernatant in a clean centrifuge bottle by decanting through a funnel lined with kit-supplied **Sieve Cloth** to filter out floating debris (**Sieve Cloth** is reusable. Rinse well under running tap and blot dry after each use).

6. **"GENECLEAN" Purification of Plasmid DNA.**
Before starting, prepare the first and second washes used in step 2 c.
a. Add 40 ml [{30}] **GLASSMILK 4G** (Resuspend **GLASSMILK 4G** by shaking first), and incubate 5 min at room temperature with occasional mixing for efficient DNA binding.

b. Spin at 600 x g for 1-2 min (2000 rpm) and discard the supernatant. (Plasmid DNA is bound to the **GLASSMILK**. The supernate contains proteins, metabolites and degraded RNA).

c. Add 50 ml user-supplied 1:1 acetone/ethanol solution (25 ml acetone + 25 ml ethanol). Gently swirl to resuspend the **GLASSMILK**/DNA complex (the pellet may be dislodged from the wall first by using a pipet). Add 50 ml of **Wash Solution 4G** (add ethanol before first use) and invert to mix. Spin as in step b. and discard the supernatant. Invert and allow to drain onto a paper towel for 1-5 minutes.

d. Prepare the Filter Unit 4G: Uncap the 50 ml **DNA Trap Tube** and place it into **Bottle**; screw a **Filter Unit 4G** onto **Bottle**. Place in cardboard stabilizer (**Bottle Holder**), and attach the filter to vacuum source. The foam insert in the bottle will exert slight upward pressure on the trap tube so that liquid exiting filter will all be contained by the **DNA Trap Tube**.

Note: The use of the **Filter Unit 4G** is an important part of this procedure and contributes significantly to the purity of the DNA which is especially important for in vitro and in vivo transfections. It also allows elution into a relatively small volume so that precipitation can be done in a single tube. Filtration time varies from 1-10 min depending on the vacuum source. A vacuum source (pump or house line) that provides 20-30 inches of Hg is optimal (›30 can damage membrane). During vacuum filtration, use the **Bottle Holder** to stabilize the filter.

e. Add 10 ml of autoclaved water and gently swirl to resuspend the **GLASSMILK**/DNA complex (it helps to dislodge pellet from centrifuge bottle wall first by using a pipet or a stirring rod). Decant suspension into filter and apply vacuum to elute DNA solution into **DNA Trap Tube**. When solution has filtered through and the matrix surface is exposed, add 4 ml of water and continue until the matrix surface is dry. Release the vacuum; unscrew and discard filter and matrix. Remove and cap **DNA Trap Tube**.

6. **Precipitate the DNA**

a. Transfer the DNA solution to a 50 ml high speed centrifuge tube. Add 560 µl of **5 M NaCl** and 11 ml of room temperature isopropanol. Incubate at -20°C for 30 minutes or more (longer times for lower yielding plasmids) and centrifuge for 15 minutes at 15,000 x g (e.g., 11,000 SS-34 or HG-4 swinging bucket rotor) and decant supernatant.

Note: The use of a swinging bucket rotor is preferred and will ensure that the DNA pellet is collected at the bottom of the tube and not partially on the wall of the tube as with a fixed angle rotor. Isopropanol pellets are often somewhat transparent and, as a result, more difficult to visualize than salt-containing ethanol pellets. To easily locate the pellet when using a fixed angle rotor, mark the tube prior to centrifugation.

b. *Optional Step:* Add 5 ml of 70-80 % ethanol and swirl the tube to wash the pellet. It is not necessary to resuspend the pellet in the wash. Spin at 15,000 x g for 5 to 10 min at 4°C and carefully decant supernatant, keeping a close watch on the pellet which is easily dislodged at this step. Pulse spin (e.g. ~3000 rpm for ‹ 1 min in a tabletop

centrifuge) and remove the last drop of ethanol with a pipet. Air dry 5 to 10 minutes at room temperature and dissolve the DNA in 0.6 to 1.2 ml of H2O or TE. (Be sure to recover the precipitated DNA that was deposited on the wall of the tube when using a fixed angle rotor). Store DNA at 4-20°C. DNA is ready for use without further manipulation.

"Modified"High Copy Protocol

Identical to High Copy Protocol except for the following:

* Use *Option 1* to process bacterial hosts that are End A+ and thus contain a higher level of endonuclease, such as HB101 and RR1.
* Use *Option 2* to prevent genomic DNA carry-over into the final purified plasmid.*

1. **Cell Lysis.**

Follow steps a through e in the High Copy Protocol above except use the volumes of reagents in brackets, as indicated.

f. Add 45 ml of isopropanol; mix and spin at 6,000 x g for 10 minutes at 4°C. Decant supernatant and drain upside down on a paper towel for 5 minutes to partially dry pellet.

g. Add 6 ml of 37°C H2O and resuspend pellet by gentle swirling and pipetting up and down. Transfer to a 50 ml centrifuge tube.

h. Add 50 µl **RNase Mixx** and incubate for 30 min at room temperature.

Option 1: Add 6 ml of 24:1 chloroform/isoamyl alcohol; mix by shaking several times, and spin at 2000 rpm for 2 minutes. Transfer upper aqueous phase to a clean 50 ml centrifuge tube and continue with *Option 2* or, transfer to a 250 or 1000 ml centrifuge bottle and continue with step 2.

Option 2: Place tube in an aluminum foil-covered boiling water bath for 6 minutes and transfer to a test tube rack in a -20°C freezer for 20 minutes. Add 9 ml of **LiCl** solution and mix. After 5 to 10 minutes incubation at room temperature, centrifuge at 15,000 x g for 5 min at 4°C. Transfer supernatant to a 250 or 1000 ml centrifuge bottle. Discard pellet which contains RNA and cellular DNA.

Continue with Step 2.

Low Copy Protocol

For isolating plasmid DNA from poorly producing plasmid/host systems. More cells are processed to increase plasmid yield. The Low Copy Protocol is the same as the "Modified" High Copy version except volumes of some reagents are increased.

1. **Cell Lysis.**

Numbers in { } are reagent quantities to be used when using Low Copy Protocol.

Follow steps a through e in the High Copy Protocol.

f. Add 90 ml of isopropanol; mix and spin at 6,000 x g for 10 minutes at 4°C. Decant supernatant and drain upside down on a paper towel for 5 minutes to partially dry pellet.

g. Add 6 ml of 37°C H2O and resuspend pellet by gentle swirling and pipetting up down. Transfer to a 50 ml centrifuge tube.

h. Add 100 µl **RNase Mixx** and incubate for 30 min at room temperature.

Continue at this point with the "Modified" High Copy Protocol.

Pause Points:

Cell pellets can be frozen for extended periods (at least 6 months) before processing. Thaw at 37°C for a few minutes and continue with step 1 b.

Isopropanol pellets in step 1 f. can be stored overnight in the refrigerator.

If DNA is dissolved in H2O, add 5 ml TE to the cuvette containing the DNA sample in H2O when measuring the OD260/280. DNA purified by the RPM 4G is free of proteins, metabolites, RNA and genomic DNA, and contains low levels of endotoxin for efficient transfection studies.