

# **RapidPURE™ Plasmid Mini 96 Kit**

*High-Throughput Plasmid Miniprep  
in a 96 well format*



## RapidPURE™ Plasmid Mini 96 Kit

*High-Throughput Plasmid Miniprep  
in a 96 well format*

### Application Manual

*Revision # 2067-999-3A01*

<b>Product Description</b>	<b># Preps</b>	<b>Cat No.</b>
RapidPURE™ Plasmid Mini 96 Kit	96 preps	2067-200
	4 x 96 preps	2067-400
	10 x 96 preps	2067-600

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## 1. RapidPURE™ Plasmid Mini 96 Kit Introduction

### 1.1 Introduction

The RapidPURE™ Plasmid Mini 96 Kit is designed to isolate up to 25 µg of supercoiled plasmid DNA from each 1.0 ml overnight bacterial culture grown in a 2.0 ml 96-well plate. The plasmid purification process employs a patented GLASSMILK®-embedded membrane contained within a *Turbo* Filter Plate. The RapidPURE™ Plasmid Mini 96 Kit is optimized for speed, ease of use, and high DNA quality.

### 1.2 Key Features & Applications

- High-throughput isolation of  $\leq 25$  µg plasmid DNA
- Each plate processed in approximately 1 hour
- No carryover of matrix into DNA solution with novel GLASSMILK®-embedded filter plate
- No possibility of cross-contamination
- Sealable 96-well collection plate for easy storage
- Plasmid DNA ready for transfection and other downstream applications
- No phenol/chloroform extraction or precipitation required
- Use with GS96 Broth for even higher yields

## 2. RapidPURE™ Plasmid Mini 96 Kit Components

### 2.1 RapidPURE™ Plasmid Mini Kit 96 Components

Component	# 2067-200 (96 preps)	2067-400 (4 x 96 preps)	# 2067-600 (10 x 96 preps)
Pre-Lysis Solution	12.5 ml	50 ml	125 ml
Alkaline Lysis Solution	25 ml	100 ml	250 ml
Neutralizing Solution	20 ml	80 ml	200 ml
Lysate Filter Plate	1	4	10
RapidPURE™ <i>Turbo</i> 96 DNA Filter Plate	1	4	10
RapidPURE™ <i>Turbo</i> 96 Binding Solution*	55 ml	220 ml	550 ml
RapidPURE™ <i>Turbo</i> 96 Wash Solution**	18 ml	72 ml	2 x 90 ml
96-Well Adhesive Seal	2	8	20
Collection Plate	1	4	10
Collection Plate Mat	1	4	10
Elution Solution***	10 ml	40 ml	100 ml

\*Solution contains guanidine thiocyanate, avoid inhalation and contact with skin

\*\*Add appropriate volume of 100% ethanol to Wash Solution and mix before first use

\*\*\*RNase/DNase/Pyrogen-free water

# RapidPURE™ Plasmid Mini 96 Kit

## 2.2 Storage of RapidPURE™ Plasmid Mini 96 Kit

The RapidPURE™ Plasmid Mini 96 Kit is shipped and stored at room temperature. It is stable for at least 1 year from the time it is shipped.

## 3. RapidPURE™ Plasmid Mini 96 Kit Protocol

### 3.1 Things to Know Before Using the RapidPURE™ Plasmid Mini 96 Kit

#### 3.1.1 Overnight Culture

Under antibiotic selection, add 1 ml CIRCLEGROW® Broth (Cat # 3000-112) to each well and inoculate with fresh bacterial colonies. Cover the plate with sterile aluminum foil and shake at 225 rpm at 37°C for 24-40 hours (better results are obtained if cells are grown for 30-40 hours). The shaking speed can be lowered to 100 rpm if using GS96, a rich media designed for optimal growth with low aeration (Cat # 3101-112). Alternative rich media like Super Broth (Cat # 3010-012) and Terrific Broth II (Cat # 3046-012) can also be used. If using LB Medium (Cat # 3002-012), grow for only ~16-24 hours to avoid processing a large number of dead cells. A lower plasmid DNA yield should be expected from standard LB Medium.

#### 3.1.2 Preparation of RapidPURE™ Turbo 96 Wash Solution

Ethanol must be added to the Wash Solution before the first use. Using the chart below, add the correct volume of 200 proof (100%) ethanol to the concentrated RapidPURE™ Turbo 96 Wash Solution provided in the kit. Invert several times to mix well. Store tightly capped at 15-30°C.

<u>Cat #</u>	<u># of preps</u>	<u>Wash Solution</u>	<u>200 proof (100%) Ethanol</u>
2067-200	96	18 ml	162 ml
2067-400	4 x 96	72 ml	648 ml
2067-600	10 x 96	2 x 90 ml	2 x 810 ml

#### 3.1.3 RapidPURE™ Plasmid Mini 96 Filter Plate

Yield: Up to 25 µg plasmid DNA per well

Volume: Up to 800 µl per well. When dispensing solutions, keep pipet tips as close as possible to the wells but outside the well space to avoid sample overflow due to solution displacement by the tips.

The RapidPURE™ Plasmid Mini 96 Filter Plate is compatible with all vacuum manifolds. The specially designed long drip director system prevents sample cross-contamination during vacuum filtration and centrifugation. The RapidPURE™ Plasmid Mini 96 Filter Plate can be used with any vacuum pump or a house vacuum, but do not use with a H<sub>2</sub>O aspirator.

# RapidPURE™ Plasmid Mini 96 Kit

## 3.1.4 Required and Suggested Materials for the RapidPURE™ Plasmid Mini 96 Kit

The RapidPURE™ Plasmid Mini 96 Kit protocol requires the use of a multichannel pipet, vortex machine with 96-well plate adapter, vacuum manifold, centrifuge/rotor for deep 96-well plates, sterile 2 ml 96-well culture plates, standard 96-well plates, and 100% ethanol.

## 3.2 RapidPURE™ Plasmid Mini 96 Kit Detailed Protocol

1. Pellet 1 ml bacterial cells grown in 2 ml 96-well culture plates by spinning at 2000 x g for 10 minutes (~3,400 rpm in IEC Centra GP8R centrifuge). Decant the supernatant by inverting the culture plate and gently tap the inverted plate on a paper towel 2-4 times to drain the remaining media.

**Note:** Residual media will not interfere with the purification process. See section 3.1.1 for information on culturing conditions.

2. COMPLETELY resuspend the cells in each well in 100 µl of Pre-Lysis Solution. Place the culture plate on a plate adapter mounted on a vortex machine and vortex at maximum speed for 10-30 seconds or until all the cells are resuspended. Complete cell resuspension is achieved if no cell clumps are visible when lifting the plate up and checking the outside bottom of the wells.

**Note:** Complete cell resuspension is essential for maximal plasmid release.

3. Add 200 µl Alkaline Lysis Solution and seal the plate with 96-Well Adhesive Seal. To seal the plate, peel the clear 96-Well Adhesive Seal from the support paper via the white tabs on either side. Place over the plate and seal with a plate roller or by hand (slide fingers 2-3 times over the entire plate surface to adequately seal the wells). Save the support paper as it can be used to vacuum seal unused plate wells in step 9.

4. Invert plate gently 3-4 times to mix.

5. Spin 2 minutes at 1000 x g at room temperature to remove remaining lysate from the cover seal (~1,000 rpm in IEC Centra GP8R centrifuge).

**Note:** Spinning is essential to prevent sample cross contamination.

6. Remove and discard the seal. IMMEDIATELY add 150 µl of Neutralizing Solution and seal the plate with a new 96-Well Adhesive Seal.

# RapidPURE™ Plasmid Mini 96 Kit

- Mix by inverting the plate 4-6 times or by vortexing at a low to intermediate speed for approximately 15 seconds. Do not remove the seal yet.

**Note:** The cell debris precipitate generated after the mixing step should be fluffy and homogenous. A large chunky and sticky precipitate could result in plasmid loss and clog the Lysate Filter Plate (step 9). If this occurs, mix by inverting the sealed plate 4-6 more times. It may be necessary to repeat the process using fewer starting cells.

**Note:** Rough handling of lysate after the addition of Neutralizing Solution will cause genomic DNA contamination. Vortexing at low speeds is safe.

- Spin the sealed plate for 5 minutes at 2000 x g at room temperature to remove remaining material from the cover seal (~3,400 rpm in IEC Centra GP8R centrifuge). Remove and discard the seal.

- Transfer the liquid (containing the cell debris) to a Lysate Filter Plate assembled into vacuum manifold with a clean, user-supplied 2 ml 96-well plate in the bottom. Keep track of orientation to avoid sample mix-up. Collect the clear supernatant by vacuum filtration for 2-5 minutes or until all the wells are dry. Discard the Lysate Filter Plate.

**Note:** It is not unusual for the lysate to start dripping from the Lysate Filter Plate soon after it is added. To avoid sample loss, add lysate to Lysate Filter Plate after it is assembled into the vacuum manifold.

**Note:** The vacuum filtration process will proceed faster if unused wells are sealed with the 96-Well Adhesive Seal SUPPORT PAPER. Sealing is automatically achieved by placing the support paper on top of unused wells and applying vacuum. Used wells should not be sealed as the filtration process will be halted.

**Note:** If a well clogs, gently stir contents with a clean 200 µl pipet tip while gently touching the membrane surface during the filtration process. A stronger vacuum source may be necessary if clogging persists.

- Add 450 µl of RapidPURE™ Turbo 96 Binding Solution to the plate containing the cleared lysate. Mix by pipetting up and down two times with a 1 ml multi-channel pipet and transfer to RapidPURE™ Turbo 96 DNA Filter Plate assembled into a vacuum manifold with a waste tray in the bottom. Keep track of orientation to avoid sample mix-up. Drain the supernatant by vacuum filtration for 2-5 minutes or until all the wells are dry.

- Add 700 µl of RapidPURE™ Turbo 96 Wash Solution to each used well (add ethanol before first use, see Section 3.1.2) and drain by vacuum filtration for 30-60 seconds. Repeat the wash step once more. After the second wash has drained, keep the vacuum on for an additional 10 minutes.

12. Remove the RapidPURE™ *Turbo* 96 DNA Filter Plate from the vacuum manifold and align on top of a standard 96-well plate. Spin at 2000 x g for 10 minutes at room temperature to remove all traces of ethanol from the membrane and drip director tips (~3,500 rpm in IEC Centra GP8R centrifuge).

**Note:** It is not necessary to secure the DNA Filter plate and standard 96-well plate as long as the combined height is less than or equal to the side wall height of the 96-well rotor.

13. Discard the standard 96-well plate used to collect trace ethanol, and align the RapidPURE™ *Turbo* 96 DNA Filter Plate on top of a Collection Plate.

14. Add 75 µl of Elution Solution DIRECTLY onto the membrane, incubate 1 minute at room temperature and spin 2 minutes at 2000 x g to collect the DNA. DNA is ready for use.

15. Seal the Collection Plate with the provided Collection Plate Mat. This is best achieved by aligning the cover over the Collection Plate and applying even pressure to seal the wells. Alternatively, a high throughput sealing device is available through Fisher Scientific (Cat# 09761150).

16. The sealed Collection Plate containing the DNA can be stored upright at 4°C or -20°C.

17. After storage, and prior to removing the Collection Plate Mat, spin the Collection Plate to remove any liquid which has condensed to avoid changes in sample concentration or sample loss. Cross contamination between samples could occur if the sealed plate has been inverted. To remove the Collection Plate Mat hold the Collection Plate securely on the bench top and pull the mat by the tab in a diagonal direction.

## 4. Troubleshooting

### 4.1 Low Plasmid DNA Recovery

Ensure that the appropriate antibiotic selection was present during overnight growth of bacterial cells.

Do not process more than 1.0 ml overnight culture. The addition of excess cells will result in inefficient lysis and the presence of heavy streaks after the lysis step and/or the presence of a “goeey” precipitate after the neutralization step. If this occurs, repeat the plasmid purification process with fewer cells.

Verify that 100% ethanol was added to the Wash Solution before first use.

Ensure that cells are completely resuspend in the Pre-Lysis Buffer.

Add Elution Solution, H<sub>2</sub>O or TE directly onto the GLASSMILK®-embedded binding membrane during the elution step, and incubate for 5 minutes before processing.

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## 4.2 RNA Contamination

In some cases, the lysis of bacterial cultures can result in an excess level of RNA that cannot be completely degraded by the RNase mixture in the Pre-Lysis Buffer. If desired, each 1 ml of Pre-Lysis Buffer can be supplemented with 5  $\mu$ l of 10 mg/ml RNaseA before use.

## 4.3 Genomic DNA Contamination

Genomic DNA contamination can occur as a result of prolonged sample exposure to the denaturing conditions of the Alkaline Lysis Solution and/or extensive shaking of the mixture following the addition of the Neutralizing Solution. Using caution at these steps can eliminate genomic DNA contamination.

## 4.4 Trouble with Downstream Applications

Incomplete removal of the Wash Solution, which contains ethanol, will result in inconsistent problems in downstream applications. It is critical to follow step 11 of the RapidPURE™ Plasmid Mini 96 Kit Comprehensive Protocol and leave the vacuum on for 10 additional minutes after the second wash to ensure removal of any residual ethanol from the DNA.

## 5. Related Products

### 5.1 Plasmid Purification Kits

<u>Purification Scale</u>	<u>Cat #</u>	<u>Kit Name</u>	<u>Size</u>
Miniprep	2066-200	RapidPURE™ Plasmid Mini Kit	60
	2066-400	RapidPURE™ Plasmid Mini Kit	120
	2066-600	RapidPURE™ Plasmid Mini Kit	300
	2069-400	Yeast RPM® Kit	100
	2000-200	MiniPrep Express™ Matrix	1,250
	2002-200	96well Prep Express	384
Midiprep	2002-400	96well Prep Express	960
	2005-200	RapidPURE™ Plasmid Midi Kit	25
	2005-400	RapidPURE™ Plasmid Midi Kit	75
	2005-600	RapidPURE™ Plasmid Midi Kit	150
Maxiprep	2076-200	RapidPURE™ Plasmid Maxi GF Kit	20
	2074-200	RapidPURE™ Plasmid Maxi GF Endo Free Kit	10
Gigaprep	2078-200	RapidPURE™ Plasmid Giga Kit	12

## 5.2 Molecular Biology Certified™ Growth Media

BIO 101® Systems is well known for providing an excellent selection of high-quality growth media in a variety of formulations. We specialize in formulations for yeast and bacterial genetics, and offer more than a thousand recipes and variations. Each product is subjected to extensive quantitative testing, and is Molecular Biology Certified™ through qualitative, molecular biology based tests such as cell density and plasmid yield. Choose from a wide variety of convenient packaging formats: Pre-mixed powders are available in capsule form, single-use pouches, or in bulk sizes. Liquid media, pre-poured plates, and custom packaging options are also available. If you prefer to make your own media, BIO 101® Systems can supply you with raw materials such as tryptones and peptones, yeast extract, a wide variety of sugars and salts, antibiotics, and other media additives.

## 5.3 MERLIN® Custom Services

Custom plasmid isolations are available in a number of sizes and grades to best fit your needs. Qbiogene's Cloning Grade is appropriate for many routine laboratory needs, such as subcloning, sequencing and PCR. For applications that require reduced levels of endotoxin, such as transfection, Research Grade preparations are available at <0.1 EU/mg of plasmid or <0.03 EU/mg of plasmid. Qbiogene also offers cGMP plasmid appropriate for clinical applications.

## 6. Product Use Limitation & Warranty

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