

# Geneaid™ Maxi Plasmid Kit & Geneaid™ Maxi Plasmid Kit (Endotoxin Free)

**PM002, PME02** (2 Preparation Sample Kit)

**PM010, PME10** (10 Preparation Kit)

**PM025, PME25** (25 Preparation Kit)

## Advantages

**Sample:** cultured bacterial cells (high-copy = 200-800 ml, low-copy = 500-1600 ml)

**Yield:** 1.0 mg of transfection grade plasmid DNA from 300 ml of cultured bacterial cells

**Format:** anion-exchange resin column, gravity flow

**Endotoxin Removal:** <0.1 EU/μg DNA verified by LAL when using PER Buffer

**Operation Time:** within 100 minutes

**Elution Volume:** 500 μl-2 ml

**Kit Storage:** dry at room temperature (15-25°C)

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## Introduction

The Geneaid™ Maxi Plasmid Kit uses pre-packed anion-exchange resin columns to purify plasmid DNA from 200-1600 ml of cultured bacterial cells. TrueBlue Lysis Buffer (an optional color indicator) is included with the kit in order to prevent common handling errors, ensuring efficient cell lysis and neutralization. A modified alkaline lysis method and RNase treatment are used to obtain clear cell lysate with minimal genomic DNA/RNA contaminants. Using an efficient gravity-flow procedure, plasmid DNA is bound to the column and contaminants are efficiently removed. The purified plasmid DNA is eluted then precipitated with isopropanol for desalting. The entire procedure can be completed without ultracentrifuges, HPLC or other toxic reagents and the purified plasmid DNA is suitable for transfection, sequencing reactions, ligation, PCR, in-vitro transcription, microinjection, restriction enzyme digestion and gene gun.

## Quality Control

The quality of the Geneaid™ Maxi Plasmid Kit is tested on a lot-to-lot basis by isolating plasmid DNA from a 400 ml overnight *E. coli* (DH5α) culture, containing plasmid pBluescript (A600 > 2 U/ml). More than 900 µg of plasmid DNA is quantified with a spectrophotometer. The purified plasmid (1 µg) is used in *EcoRI* digestion and analyzed by electrophoresis.

## Kit Components

Component	PM002/PME02	PM010/PME10	PM025/PME25
PM1 Buffer <sup>1</sup>	25 ml	110 ml	275 ml
TrueBlue Lysis Buffer	250 µl	1.5 ml	1.5 ml x 2
PER Buffer (PME Only)	8 ml	40 ml	100 ml
PM2 Buffer <sup>2</sup>	25 ml	110 ml	275 ml
PM3 Buffer	25 ml	110 ml	275 ml
PEQ Buffer	25 ml	130 ml	275 ml
PMC Buffer	65 ml	120 ml x 1 240 ml x 1	240 ml x 1 550 ml x 1
PEL Buffer	25 ml	130 ml	130 ml x 1 220 ml x 1
RNase A (50 mg/ml)	Added	200 µl	550 µl
Plasmid Maxi Columns	2	10	25

<sup>1</sup>For PM010, PME10, PM025, PME25 add provided RNase A to PM1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. PM1 Buffer and RNase A mixture should be stored at 2-8°C for up to 6 months. For PM002, PME02 samples, RNase A was already added to PM1 Buffer.

<sup>2</sup>If precipitates have formed in PM2 Buffer, warm in a 37°C water bath, followed by gentle shaking to dissolve.



**During the procedure, always wear a lab coat, disposable gloves, and protective goggles.**

## Quick Protocol Diagram



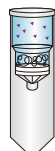
Harvest cultured bacterial cells by centrifuge to form a cell pellet, followed by resuspension



Lyse bacterial cells (optional color indicator will turn blue when lysis is successful)



Neutralize suspension (optional color indicator will become clear when neutralization is successful). When using PME02, PME10 and PME25, neutralization is followed by PER Buffer treatment to remove endotoxin.



DNA binding to silica resin while contaminants remain suspended



Wash (removal of contaminants while DNA remains bound to silica resin)



Elution and precipitation of pure plasmid DNA which is ready for subsequent reactions

## Recommended Culture Volume

Plasmid Type	Pellet Wet Weight	OD600 = 2	OD600 = 4	OD600 = 6
High-copy number	3 g	800 ml	400 ml	250 ml
Low-copy number	6 g	1600 ml	800 ml	500 ml

NOTE: For a higher yield, increase lysis buffer volumes by 1.5 times when using more than 3 g of cultured bacterial pellet. In this case, additional lysis buffer can be purchased from Geneaid.

# Geneaid™ Maxi Plasmid Kit Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

## IMPORTANT BEFORE USE!

1. For PM010 and PM025 add provided RNase A to PM1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. PM1 Buffer and RNase A mixture should be stored at 2-8°C for up to 6 months. For PM002 samples, RNase A was already added to PM1 Buffer.
2. If precipitates have formed in PM2 Buffer, warm in a 37°C water bath followed by gentle shaking to dissolve.

### Additional Requirements

50 ml centrifuge tubes, isopropanol, 75% ethanol, TE or ddH<sub>2</sub>O



*PW Buffer has been replaced with PMC Buffer. DO NOT USE leftover PW Buffer. **ONLY USE PMC Buffer with this kit.***

## Protocol Procedure With Color Indicator

### 1. Harvesting

Transfer **cultured bacterial cells** to a 50 ml centrifuge tube or a 250 ml centrifuge bottle. Centrifuge at  $\geq 3,000 \times g$  for 15 minutes at room temperature to form a cell pellet. Discard the supernatant completely. Use a narrow pipette tip to ensure the supernatant is completely removed. Repeat the Harvesting step as required for 200-800 ml of high-copy or 500-1600 ml of low-copy cultured bacterial cells using the same 50 ml centrifuge tube or 250 ml centrifuge bottle.

NOTE: Using 2 OD600 - 6 OD600 units of bacterial culture is recommended. Do not use overgrown bacterial cultures ( $\leq 16$  hours incubated in a flask at 37°C with 150-180 rpm shaking). Use fresh bacterial cultures only. Solid and liquid medium (i.e. LB medium) should contain an antibiotic such as ampicillin.

### 2. Equilibration

During centrifugation, place a **Plasmid Maxi Column** in a new 50 ml centrifuge tube. Equilibrate the **Plasmid Maxi Column** by adding **10 ml of PEQ Buffer**. Allow the column to empty completely by gravity flow. Discard the flow-through and place the **Plasmid Maxi Column** back in the 50 ml centrifuge tube then set it aside for Step 6.

### 3. Resuspension

Add **10 ml of PM1 Buffer (make sure RNase A was added) and 100  $\mu$ l of TrueBlue Lysis Buffer** to a new 50 ml centrifuge tube. Mix by shaking gently.

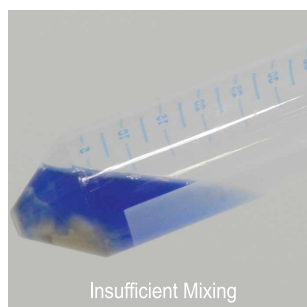
NOTE: If the cell pellet is  $> 3$  g (wet weight), mix 15 ml of PM1 Buffer and 150  $\mu$ l of TrueBlue Lysis Buffer. It is normal for precipitates to form after mixing TrueBlue Lysis Buffer with PM1 Buffer.

Transfer the mixture to the 50 ml centrifuge tube or the 250 ml centrifuge bottle containing the cell pellet. Resuspend the cell pellet by vortex, pipette or scraping the tube across the top of a 1.5 ml microcentrifuge tube rack until all traces of the cell pellet have been completely dissolved. If using a 250 ml centrifuge bottle, transfer the resuspended sample to a new 50 ml centrifuge tube.

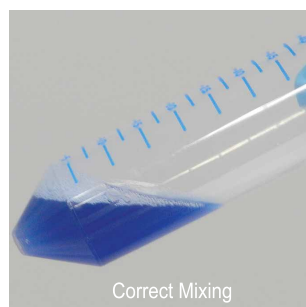
## 4. Cell Lysis

Add **10 ml of PM2 Buffer** to the resuspended sample then mix gently by inverting the tube 10 times. Close PM2 Buffer bottle immediately after use to avoid CO<sub>2</sub> acidification. Do not vortex to avoid shearing the genomic DNA. Let stand at room temperature for at least 2 minutes to ensure the lysate is homogeneous. Do not exceed 5 minutes.

NOTE: If the cell pellet is >3 g (wet weight), add 15 ml of PM2 Buffer. After adding PM2 Buffer, any precipitates will be completely dissolved and the color of the suspension will become blue. If the suspension contains colorless regions or brownish cell clumps, continue mixing until the suspension is completely blue.



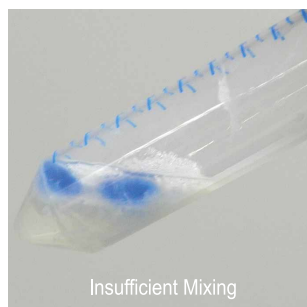
If colorless regions or brownish cell clumps are present, continue mixing until the suspension is completely blue.



## 5. Neutralization

Add **10 ml of PM3 Buffer** and mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Centrifuge at  $\geq 3,000 \times g$  for 20 minutes at room temperature.

NOTE: If the cell pellet is >3 g (wet weight), add 15 ml of PM3 Buffer. After adding PM3 Buffer, the suspension will become colorless. If blue regions remain in the suspension, continue mixing until it becomes colorless.



If blue regions are present, continue mixing until the suspension is completely colorless.



## 6. DNA Binding

Transfer the supernatant to the equilibrated **Plasmid Maxi Column**. Allow the column to empty completely by gravity flow. Discard the flow-through then place the **Plasmid Maxi Column** back in the 50 ml centrifuge tube.

## 7. Wash

Wash the **Plasmid Maxi Column** by adding **30 ml of PMC Buffer** and allow the column to empty completely by gravity flow then discard the flow-through.

## 8. Elution

Place the **Plasmid Maxi Column** in a clean 50 ml centrifuge tube then add **12 ml of PEL Buffer** to elute the DNA by gravity flow. Discard the **Plasmid Maxi Column** once it has emptied completely.

## 9. DNA Precipitation

Add **9 ml (0.75 volumes) of isopropanol** to the eluted DNA from Step 8. Mix the tube completely by inverting then centrifuge at  $\geq 3,000 \times g$  for 20 minutes (preferably at  $15,000 \times g$  for 30 minutes) at  $4^{\circ}\text{C}$ . Carefully remove the supernatant then wash the DNA pellet with **5 ml of 75% ethanol**. Centrifuge at  $\geq 3,000 \times g$  for 5 minutes (preferably at  $15,000 \times g$  for 10 minutes) at  $4^{\circ}\text{C}$ . Carefully remove the supernatant then air-dry the DNA pellet for 10 minutes. Once the DNA pellet is dry, add **500  $\mu\text{l}$ –2 ml (or a suitable volume) of TE<sup>1</sup> or water<sup>2</sup>** then place the tube in a  $60^{\circ}\text{C}$  water bath for 5-10 minutes to dissolve the DNA pellet.

NOTE: Following both centrifugation steps, extra caution is needed when removing the supernatant to avoid contacting the DNA pellet.

<sup>1</sup>Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications.

<sup>2</sup>If using water, ensure the water pH is  $\geq 8.0$ . ddH<sub>2</sub>O should be fresh as ambient CO<sub>2</sub> can quickly cause acidification.

**IMPORTANT BEFORE USE!**

1. For PM010 and PM025 add provided RNase A to PM1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. PM1 Buffer and RNase A mixture should be stored at 2-8°C for up to 6 months. For PM002 samples, RNase A was already added to PM1 Buffer.

2. If precipitates have formed in PM2 Buffer, warm in a 37°C water bath then shake gently to dissolve.

**Additional Requirements**

50 ml centrifuge tubes, isopropanol, 75% ethanol, TE or ddH<sub>2</sub>O



*PW Buffer has been replaced with PMC Buffer. DO NOT USE leftover PW Buffer. **ONLY USE PMC Buffer with this kit.***

## Protocol Procedure Without Color Indicator

### 1. Harvesting

Transfer **cultured bacterial cells** to a 50 ml centrifuge tube or a 250 ml centrifuge bottle. Centrifuge at  $\geq 3,000 \times g$  for 15 minutes at room temperature to form a cell pellet. Discard the supernatant completely. Use a narrow pipette tip to ensure the supernatant is completely removed. Repeat the Harvesting step as required for 200-800 ml of high-copy or 500-1600 ml of low-copy cultured bacterial cells using the same 50 ml tube or 250 ml centrifuge bottle.

NOTE: Using 2 OD<sub>600</sub> - 6 OD<sub>600</sub> units of bacterial culture is recommended. Do not use overgrown bacterial cultures ( $\leq 16$  hours incubated in a flask at 37°C with 150-180 rpm shaking). Use fresh bacterial cultures only. Solid and liquid medium (i.e. LB medium) should contain an antibiotic such as ampicillin.

### 2. Equilibration

During centrifugation, place a **Plasmid Maxi Column** in a new 50 ml centrifuge tube. Equilibrate the **Plasmid Maxi Column** by adding **10 ml of PEQ Buffer**. Allow the column to empty completely by gravity flow. Discard the flow-through and place the **Plasmid Maxi Column** back in the 50 ml centrifuge tube then set it aside for Step 6.

### 3. Resuspension

Add **10 ml of PM1 Buffer (make sure RNase A was added)**. Resuspend the cell pellet by vortex, pipette or scraping the tube across the top of a 1.5 ml microcentrifuge tube rack until all traces of the cell pellet have been completely dissolved. If using a 250 ml centrifuge bottle, transfer the resuspended sample to a new 50 ml centrifuge tube.

NOTE: If the cell pellet is  $>3$  g (wet weight), add 15 ml of PM1 Buffer.

### 4. Cell Lysis

Add **10 ml of PM2 Buffer** to the resuspended sample then mix gently by inverting the tube 10 times. Close PM2 Buffer bottle immediately after use to avoid CO<sub>2</sub> acidification. Do not vortex to avoid shearing the genomic DNA. Let stand at room temperature for at least 2 minutes to ensure the lysate is homogeneous. Do not exceed 5 minutes.

NOTE: If the cell pellet is  $>3$  g (wet weight), add 15 ml of PM2 Buffer.

## 5. Neutralization

Add **10 ml of PM3 Buffer** then mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Centrifuge at  $\geq 3,000 \times g$  for 20 minutes at room temperature.

NOTE: If the cell pellet is  $>3$  g (wet weight), add 15 ml of PM3 Buffer.

## 6. DNA Binding

Transfer the supernatant to the equilibrated **Plasmid Maxi Column**. Allow the column to empty completely by gravity flow. Discard the flow-through then place the **Plasmid Maxi Column** back in the 50 ml centrifuge tube.

## 7. Wash

Wash the **Plasmid Maxi Column** by adding **30 ml of PMC Buffer** and allow the column to empty completely by gravity flow then discard the flow-through.

## 8. Elution

Place the **Plasmid Maxi Column** in a clean 50 ml centrifuge tube then add **12 ml of PEL Buffer** to elute the DNA by gravity flow. Discard the **Plasmid Maxi Column** once it has emptied completely.

## 9. DNA Precipitation

Add **9 ml (0.75 volumes) of isopropanol** to the eluted DNA from Step 8. Mix the tube completely by inverting then centrifuge at  $\geq 3,000 \times g$  for 20 minutes (preferably at  $15,000 \times g$  for 30 minutes) at  $4^{\circ}\text{C}$ . Carefully remove the supernatant then wash the DNA pellet with **5 ml of 75% ethanol**. Centrifuge at  $\geq 3,000 \times g$  for 5 minutes (preferably at  $15,000 \times g$  for 10 minutes) at  $4^{\circ}\text{C}$ . Carefully remove the supernatant then air-dry the DNA pellet for 10 minutes. Once the DNA pellet is dry, add **500  $\mu\text{l}$ –2 ml (or a suitable volume) of TE<sup>1</sup> or water<sup>2</sup>** then place the tube in a  $60^{\circ}\text{C}$  water bath for 5-10 minutes to dissolve the DNA pellet.

NOTE: Following both centrifugation steps, extra caution is needed when removing the supernatant to avoid contacting the DNA pellet.

<sup>1</sup>Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications.

<sup>2</sup>If using water, ensure the water pH is  $\geq 8.0$ . ddH<sub>2</sub>O should be fresh as ambient CO<sub>2</sub> can quickly cause acidification.



# Geneaid™ Maxi Plasmid Kit (Endotoxin Free) Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

## IMPORTANT BEFORE USE!

1. For PME10 and PME25 add provided RNase A to PM1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. PM1 Buffer and RNase A mixture should be stored at 2-8°C for up to 6 months. For PME02 samples, RNase A was already added to PM1 Buffer.
2. If precipitates have formed in PM2 Buffer, warm in a 37°C water bath then shake gently to dissolve.

### Additional Requirements

50 ml centrifuge tubes, isopropanol, 75% ethanol, TE or ddH<sub>2</sub>O



*PW Buffer has been replaced with PMC Buffer. DO NOT USE leftover PW Buffer. **ONLY USE PMC Buffer with this kit.***

## Protocol Procedure With Color Indicator

### 1. Harvesting

Transfer **cultured bacterial cells** to a 50 ml centrifuge tube or a 250 ml centrifuge bottle. Centrifuge at  $\geq 3,000 \times g$  for 15 minutes at room temperature to form a cell pellet. Discard the supernatant completely. Use a narrow pipette tip to ensure the supernatant is completely removed. Repeat the Harvesting step as required for 200-800 ml of high-copy or 500-1600 ml of low-copy cultured bacterial cells using the same 50 ml tube or 250 ml centrifuge bottle.

NOTE: Using 2 OD<sub>600</sub> - 6 OD<sub>600</sub> units of bacterial culture is recommended. Do not use overgrown bacterial cultures ( $\leq 16$  hours incubated in a flask at 37°C with 150-180 rpm shaking). Use fresh bacterial cultures only. Solid and liquid medium (i.e. LB medium) should contain an antibiotic such as ampicillin.

### 2. Equilibration

During centrifugation, place a **Plasmid Maxi Column** in a new 50 ml centrifuge tube. Equilibrate the **Plasmid Maxi Column** by adding **10 ml of PEQ Buffer**. Allow the column to empty completely by gravity flow. Discard the flow-through and place the **Plasmid Maxi Column** back in the 50 ml centrifuge tube then set it aside for Step 7.

### 3. Resuspension

Add **10 ml of PM1 Buffer (make sure RNase A was added)** and **100  $\mu$ l of TrueBlue Lysis Buffer** to a new 50 ml centrifuge tube. Mix by shaking gently.

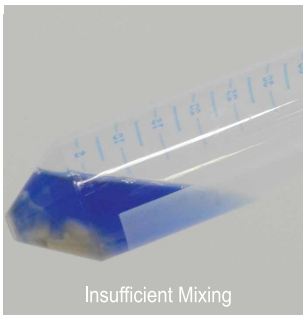
NOTE: If the cell pellet is  $> 3$  g (wet weight), mix 15 ml of PM1 Buffer and 150  $\mu$ l of TrueBlue Lysis Buffer. It is normal for precipitates to form after mixing TrueBlue Lysis Buffer with PM1 Buffer.

Transfer the mixture to the 50 ml centrifuge tube or the 250 ml centrifuge bottle containing the cell pellet. Resuspend the cell pellet by vortex, pipette or scraping the tube across the top of a 1.5 ml microcentrifuge tube rack until all traces of the cell pellet have been completely dissolved. If using a 250 ml centrifuge bottle, transfer the resuspended sample to a new 50 ml centrifuge tube.

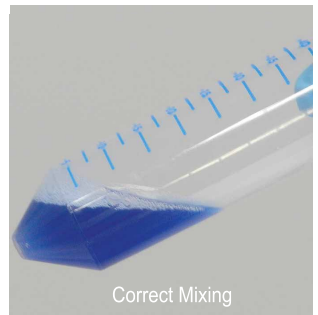
## 4. Cell Lysis

Add **10 ml of PM2 Buffer** to the resuspended sample then mix gently by inverting the tube 10 times. Close PM2 Buffer bottle immediately after use to avoid CO<sub>2</sub> acidification. Do not vortex to avoid shearing the genomic DNA. Let stand at room temperature for at least 2 minutes to ensure the lysate is homogeneous. Do not exceed 5 minutes.

NOTE: If the cell pellet is >3 g (wet weight), add 15 ml of PM2 Buffer. After adding PM2 Buffer, any precipitates will be completely dissolved and the color of the suspension will become blue. If the suspension contains colorless regions or brownish cell clumps, continue mixing until the suspension is completely blue.



If colorless regions or brownish cell clumps are present, continue mixing until the suspension is completely blue.



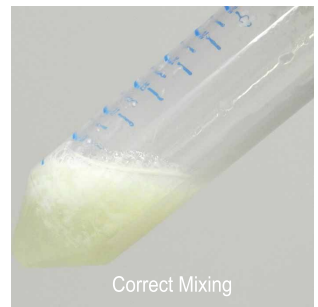
## 5. Neutralization

Add **10 ml of PM3 Buffer** and mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Centrifuge at  $\geq 3,000 \times g$  for 20 minutes at room temperature.

NOTE: If the cell pellet is >3 g (wet weight), add 15 ml of PM3 Buffer. After adding PM3 Buffer, the suspension will become colorless. If blue regions remain in the suspension, continue mixing until it becomes colorless.



If blue regions are present, continue mixing until the suspension is completely colorless.



## 6. Endotoxin Removal

NOTE: Invert PER Buffer bottle 3-5 times immediately prior to use.

Transfer the supernatant to a clean 50 ml centrifuge tube. Add **3 ml of PER Buffer** then mix by inverting 5-10 times. Incubate on ice for 30 minutes.

NOTE: Following PER Buffer addition, the mixture will become cloudy.

## 7. DNA Binding

Following ice incubation, **transfer the mixture** to the equilibrated **Plasmid Maxi Column**. Allow the column to empty completely by gravity flow. Discard the flow-through then place the **Plasmid Maxi Column** back in the 50 ml centrifuge tube.

## 8. Wash

Wash the **Plasmid Maxi Column** by adding **30 ml of PMC Buffer** and allow the column to empty completely by gravity flow then discard the flow-through.

## 9. Elution

Place the **Plasmid Maxi Column** in a clean 50 ml centrifuge tube then add **12 ml of PEL Buffer** to elute the DNA by gravity flow. Discard the **Plasmid Maxi Column** once it has emptied completely.

## 10. DNA Precipitation

Add **9 ml (0.75 volumes) of isopropanol** to the eluted DNA from Step 9. Mix the tube completely by inverting then centrifuge at  $\geq 3,000 \times g$  for 20 minutes (preferably at  $15,000 \times g$  for 30 minutes) at  $4^{\circ}\text{C}$ . Carefully remove the supernatant then wash the DNA pellet with **5 ml of 75% ethanol**. Centrifuge at  $\geq 3,000 \times g$  for 5 minutes (preferably at  $15,000 \times g$  for 10 minutes) at  $4^{\circ}\text{C}$ . Carefully remove the supernatant then air-dry the DNA pellet for 10 minutes. Once the DNA pellet is dry, add **500  $\mu\text{l}$ –2 ml (or a suitable volume) of TE<sup>1</sup> or water<sup>2</sup>** then place the tube in a  $60^{\circ}\text{C}$  water bath for 5-10 minutes to dissolve the DNA pellet.

NOTE: Following both centrifugation steps, extra caution is needed when removing the supernatant to avoid contacting the DNA pellet.

<sup>1</sup>Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications.

<sup>2</sup>If using water, ensure the water pH is  $\geq 8.0$ . ddH<sub>2</sub>O should be fresh as ambient CO<sub>2</sub> can quickly cause acidification.

**IMPORTANT BEFORE USE!**

1. For PME10 and PME25 add provided RNase A to PM1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. PM1 Buffer and RNase A mixture should be stored at 2-8°C for up to 6 months. For PME02 samples, RNase A was already added to PM1 Buffer.

2. If precipitates have formed in PM2 Buffer, warm in a 37°C water bath then shake gently to dissolve.

**Additional Requirements**

50 ml centrifuge tubes, isopropanol, 75% ethanol, TE or ddH<sub>2</sub>O



*PW Buffer has been replaced with PMC Buffer. DO NOT USE leftover PW Buffer. **ONLY USE PMC Buffer with this kit.***

## Protocol Procedure Without Color Indicator

### 1. Harvesting

Transfer **cultured bacterial cells** to a 50 ml centrifuge tube or a 250 ml centrifuge bottle. Centrifuge at  $\geq 3,000 \times g$  for 15 minutes at room temperature to form a cell pellet. Discard the supernatant completely. Use a narrow pipette tip to ensure the supernatant is completely removed. Repeat the Harvesting step as required for 200-800 ml of high-copy or 500-1600 ml of low-copy cultured bacterial cells using the same 50 ml tube or 250 ml centrifuge bottle.

NOTE: Using 2 OD<sub>600</sub> - 6 OD<sub>600</sub> units of bacterial culture is recommended. Do not use overgrown bacterial cultures ( $\leq 16$  hours incubated in a flask at 37°C with 150-180 rpm shaking). Use fresh bacterial cultures only. Solid and liquid medium (i.e. LB medium) should contain an antibiotic such as ampicillin.

### 2. Equilibration

During centrifugation, place a **Plasmid Maxi Column** in a new 50 ml centrifuge tube. Equilibrate the **Plasmid Maxi Column** by adding **10 ml of PEQ Buffer**. Allow the column to empty completely by gravity flow. Discard the flow-through and place the **Plasmid Maxi Column** back in the 50 ml centrifuge tube then set it aside for Step 7.

### 3. Resuspension

Add **10 ml of PM1 Buffer (make sure RNase A was added)**. Resuspend the cell pellet by vortex, pipette or scraping the tube across the top of a 1.5 ml microcentrifuge tube rack until all traces of the cell pellet have been completely dissolved. If using a 250 ml centrifuge bottle, transfer the resuspended sample to a new 50 ml centrifuge tube.

NOTE: If the cell pellet is  $>3$  g (wet weight), add 15 ml of PM1 Buffer.

### 4. Cell Lysis

Add **10 ml of PM2 Buffer** to the resuspended sample then mix gently by inverting the tube 10 times. Close PM2 Buffer bottle immediately after use to avoid CO<sub>2</sub> acidification. Do not vortex to avoid shearing the genomic DNA. Let stand at room temperature for at least 2 minutes to ensure the lysate is homogeneous. Do not exceed 5 minutes.

NOTE: If the cell pellet is  $>3$  g (wet weight), add 15 ml of PM2 Buffer.

## 5. Neutralization

Add **10 ml of PM3 Buffer** then mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Centrifuge at  $\geq 3,000 \times g$  for 20 minutes at room temperature.

NOTE: If the cell pellet is  $>3$  g (wet weight), add 15 ml of PM3 Buffer.

## 6. Endotoxin Removal

NOTE: Invert PER Buffer bottle 3-5 times immediately prior to use.

Transfer the supernatant to a clean 50 ml centrifuge tube. Add **3 ml of PER Buffer** then mix by inverting 5-10 times. Incubate on ice for 30 minutes.

NOTE: Following PER Buffer addition, the mixture will become cloudy.

## 7. DNA Binding

Following ice incubation, **transfer the mixture** to the equilibrated **Plasmid Maxi Column**. Allow the column to empty completely by gravity flow. Discard the flow-through then place the **Plasmid Maxi Column** back in the 50 ml centrifuge tube.

## 8. Wash

Wash the **Plasmid Maxi Column** by adding **30 ml of PMC Buffer** and allow the column to empty completely by gravity flow then discard the flow-through.

## 9. Elution

Place the **Plasmid Maxi Column** in a clean 50 ml centrifuge tube then add **12 ml of PEL Buffer** to elute the DNA by gravity flow. Discard the **Plasmid Maxi Column** once it has emptied completely.

## 10. DNA Precipitation

Add **9 ml (0.75 volumes) of isopropanol** to the eluted DNA from Step 9. Mix the tube completely by inverting then centrifuge at  $\geq 3,000 \times g$  for 20 minutes (preferably at  $15,000 \times g$  for 30 minutes) at  $4^{\circ}\text{C}$ . Carefully remove the supernatant then wash the DNA pellet with **5 ml of 75% ethanol**. Centrifuge at  $\geq 3,000 \times g$  for 5 minutes (preferably at  $15,000 \times g$  for 10 minutes) at  $4^{\circ}\text{C}$ . Carefully remove the supernatant then air-dry the DNA pellet for 10 minutes. Once the DNA pellet is dry, add **500  $\mu\text{l}$ –2 ml (or a suitable volume) of TE<sup>1</sup> or water<sup>2</sup>** then place the tube in a  $60^{\circ}\text{C}$  water bath for 5-10 minutes to dissolve the DNA pellet.

NOTE: Following both centrifugation steps, extra caution is needed when removing the supernatant to avoid contacting the DNA pellet.

<sup>1</sup>Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications.

<sup>2</sup>If using water, ensure the water pH is  $\geq 8.0$ . ddH<sub>2</sub>O should be fresh as ambient CO<sub>2</sub> can quickly cause acidification.

# Troubleshooting



## Low Yield

### **Incomplete buffer preparation.**

For PM010, PME10, PM025, PME25 add provided RNase A to PM1 Buffer then mix by shaking for a few seconds. Check the box on the bottle then store at 2-8°C for up to 6 months. For PM002 and PME02 samples, RNase A was already added to PM1 Buffer. If precipitates have formed in PM2 Buffer, warm in a 37°C water bath followed by gentle shaking to dissolve.

### **Incomplete cell culture preparation.**

Use flasks which are at least 3 times the volume of the culture medium to provide an oxygen rich culture condition. Solid and liquid medium should contain antibiotics. Do not use overgrown bacterial cultures ( $\geq 16$  hours incubated in a flask at 37°C with 150-180 rpm shaking).

### **Culture growth medium was not removed completely.**

Following centrifugation in the Harvesting step, use a narrow pipette tip to ensure the supernatant is completely removed.

### **Cell pellet was not resuspended completely.**

Resuspend the cell pellet completely by vortex or pipette.

### **Bacterial cells were not lysed completely.**

Using 2 OD600 - 6 OD600 units of bacterial culture is recommended.

When using TrueBlue Lysis Buffer: Following PM2 Buffer addition, the color of the suspension will become blue. If the suspension contains colorless regions or brownish cell clumps, continue mixing until the suspension is completely blue. Do not vortex to avoid shearing the genomic DNA.

### **Bacterial cells were not neutralized completely.**

When using TrueBlue Lysis Buffer: Following PM3 Buffer addition, the suspension will become colorless. If blue regions remain in the suspension, continue mixing until it becomes colorless. Do not vortex to avoid shearing the genomic DNA.

### **Incorrect DNA Rehydration.**

If using water to dissolve the DNA pellet, ensure the water pH is  $\geq 8.0$ . ddH<sub>2</sub>O should be fresh as ambient CO<sub>2</sub> can quickly cause acidification. After adding TE Buffer or ddH<sub>2</sub>O, be sure the DNA pellet is completely immersed. Incubate at 60°C for at least 10 minutes to facilitate DNA pellet dissolution.

## Eluted DNA Does Not Perform Well In Downstream Applications

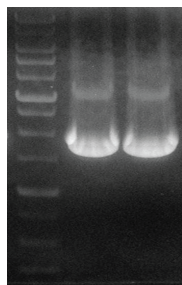
### RNA contamination.

Add provided RNase A to PM1 Buffer then mix by shaking for a few seconds. Check the box on the bottle then store at 2-8°C for up to 6 months.

### Genomic DNA contamination.

Do not use overgrown bacterial cultures. Use only fresh cultures as they will contain less genomic DNA than old cultures. During PM2 and PM3 Buffer addition, mix gently to prevent

## Geneaid™ Maxi Plasmid Kit Functional Test Data



**Figure 1.** Plasmid DNA was extracted using the Geneaid™ Maxi Plasmid Kit. The purified supercoiled Plasmid DNA [300 ml overnight *E. coli* (DH5α) culture, containing a 3 kb plasmid pBluescript (A600 > 2 U/ml, OD600 = 4.0)], was analyzed by electrophoresis on a 1% agarose gel.

M = Geneaid 1 Kb DNA Ladder

Test	DNA Conc.	260/280	260/230	Yield
1	1012.2 µg/ml	1.87	2.25	1.0 mg
2	1015.1 µg/ml	1.87	2.29	1.0 mg

**Geneaid**

