Geneaid™ Midi Plasmid Kit &
Geneaid™ Midi Plasmid Kit (Endotoxin Free)

PI002, PIE02 (2 Preparation Sample Kit)
PI025, PIE25 (25 Preparation Kit)

Advantages
Sample: cultured bacterial cells (high-copy = 50-200 ml, low-copy = 100-300 ml)
Yield: >400 µg of transfection grade plasmid DNA from 100 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 120 minutes (only 80 minutes when combined with the Presto™ Plasmid Concentrator)
Elution Volume: 500 µl-2 ml
Kit Storage: dry at room temperature (15-25°C)

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Introduction
The Geneaid™ Midi Plasmid Kit uses pre-packed anion-exchange resin columns to purify plasmid DNA from 50-300 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™ Midi Plasmid Kit is tested on a lot-to-lot basis by isolating plasmid DNA from a 100 ml overnight *E. coli* (DH5α) culture, containing plasmid pBluescript (A600 > 2 U/ml). More than 400 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

<table>
<thead>
<tr>
<th>Component</th>
<th>PI002/PIE02</th>
<th>PI025/PIE25</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM1 Buffer</td>
<td>10 ml</td>
<td>110 ml</td>
</tr>
<tr>
<td>PM2 Buffer</td>
<td>10 ml</td>
<td>110 ml</td>
</tr>
<tr>
<td>PM3 Buffer</td>
<td>10 ml</td>
<td>110 ml</td>
</tr>
<tr>
<td>PER Buffer (PIE02, PIE25 Only)</td>
<td>4 ml</td>
<td>40 ml</td>
</tr>
<tr>
<td>PEQ Buffer</td>
<td>12 ml</td>
<td>130 ml</td>
</tr>
<tr>
<td>PW Buffer</td>
<td>30 ml</td>
<td>120 ml x 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>240 ml x 1</td>
</tr>
<tr>
<td>PEL Buffer</td>
<td>25 ml</td>
<td>220 ml</td>
</tr>
<tr>
<td>RNase A (50 mg/ml)</td>
<td>Added</td>
<td>200 µl</td>
</tr>
<tr>
<td>Plasmid Midi Columns</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>TrueBlue Lysis Buffer</td>
<td>150 µl</td>
<td>1.5 ml</td>
</tr>
</tbody>
</table>

1For PI025, PIE25 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 PI002, PIE02 samples, RNase A was already added to PM1 Buffer.

2If 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 PIE02 and PIE25, 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 Volumes

<table>
<thead>
<tr>
<th>Plasmid Type</th>
<th>Pellet Wet Weight</th>
<th>OD600 = 2</th>
<th>OD600 = 4</th>
<th>OD600 = 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-copy number</td>
<td>0.75 g</td>
<td>200 ml</td>
<td>100 ml</td>
<td>66 ml</td>
</tr>
<tr>
<td>Low-copy number</td>
<td>1.12 g</td>
<td>300 ml</td>
<td>150 ml</td>
<td>100 ml</td>
</tr>
</tbody>
</table>
Geneaid™ Midi Plasmid Kit Protocol

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

IMPORTANT BEFORE USE!

1. For PI025 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 PI002 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 ddH2O

Protocol Procedure With Color Indicator

1. Harvesting

Transfer cultured bacterial cells to a 50 ml centrifuge tube then centrifuge at ≥3,000 x 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 up to 200 ml of high-copy or 100-300 ml of low-copy cultured bacterial cells using the same 50 ml centrifuge tube.

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

2. Equilibration

During centrifugation, place a Plasmid Midi Column in a new 50 ml centrifuge tube. Equilibrate the Plasmid Midi Column by adding 5 ml of PEQ Buffer. Allow the column to empty completely by gravity flow. Discard the flow-through and place the Plasmid Midi Column back in the 50 ml centrifuge tube then set it aside for Step 6.

3. Resuspension

Add 4 ml of PM1 Buffer (make sure RNase A was added) and 40 µl of TrueBlue Lysis Buffer to a new 50 ml centrifuge tube. Mix by shaking gently.

NOTE: It is normal for precipitates to form after mixing TrueBlue Lysis Buffer with PM1 Buffer. Transfer the mixture to the 50 ml centrifuge tube 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.
4. Cell Lysis

Add **4 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 CO₂ 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: 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.

5. Neutralization

Add **4 ml of PM3 Buffer** and mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Centrifuge at ≥3,000 x g for 20 minutes at room temperature.

NOTE: After adding PM3 Buffer, the suspension will become colorless. If blue regions remain in the suspension, continue mixing until it becomes colorless.
6. DNA Binding
Transfer the supernatant to the equilibrated Plasmid Midi Column. Allow the column to empty completely by gravity flow. Discard the flow-through then place the Plasmid Midi Column back in the 50 ml centrifuge tube.

7. Wash
Wash the Plasmid Midi Column by adding 12 ml of PW Buffer and allow the column to empty completely by gravity flow then discard the flow-through.

8. Elution
Place the Plasmid Midi Column in a clean 50 ml centrifuge tube then add 8 ml of PEL Buffer to elute the DNA by gravity flow. Discard the Plasmid Midi Column once it has emptied completely.

9. DNA Precipitation
Add 6 ml (0.75 volumes) of isopropanol to the eluted DNA from Step 8. Mix the tube completely by inverting then centrifuge at ≥3,000 x g for 20 minutes (preferably at 15,000 x g for 30 minutes) at 4°C. Carefully remove the supernatant then wash the DNA pellet with 5 ml of 75% ethanol. Centrifuge at ≥3,000 x g for 5 minutes (preferably at 15,000 x g for 10 minutes) at 4°C. Carefully remove the supernatant then air-dry the DNA pellet for 10 minutes. Once the DNA pellet is dry, add 500 µl–2 ml (or a suitable volume) of TE¹ or water² then place the tube in a 60°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.

¹Using 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.

²If using water, ensure the water pH is ≥8.0. ddH₂O should be fresh as ambient CO₂ can quickly cause acidification.
IMPORTANT BEFORE USE!

1. For PI025 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 PI002 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 ddH2O

Protocol Procedure Without Color Indicator

1. Harvesting
Transfer cultured bacterial cells to a 50 ml centrifuge tube then centrifuge at ≥3,000 x 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 up to 200 ml of high-copy or 100-300 ml of low-copy cultured bacterial cells using the same 50 ml centrifuge tube.

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

2. Equilibration
During centrifugation, place a Plasmid Midi Column in a new 50 ml centrifuge tube. Equilibrate the Plasmid Midi Column by adding 5 ml of PEQ Buffer. Allow the column to empty completely by gravity flow. Discard the flow-through and place the Plasmid Midi Column back in the 50 ml centrifuge tube then set it aside for Step 6.

3. Resuspension
Add 4 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.

4. Cell Lysis
Add 4 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 CO2 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.
5. Neutralization
Add 4 ml of PM3 Buffer and mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Centrifuge at ≥3,000 x g for 20 minutes at room temperature.

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

7. Wash
Wash the Plasmid Midi Column by adding 12 ml of PW Buffer and allow the column to empty completely by gravity flow then discard the flow-through.

8. Elution
Place the Plasmid Midi Column in a clean 50 ml centrifuge tube then add 8 ml of PEL Buffer to elute the DNA by gravity flow. Discard the Plasmid Midi Column once it has emptied completely.

9. DNA Precipitation
Add 6 ml (0.75 volumes) of isopropanol to the eluted DNA from Step 8. Mix the tube completely by inverting then centrifuge at ≥3,000 x g for 20 minutes (preferably at 15,000 x g for 30 minutes) at 4ºC. Carefully remove the supernatant then wash the DNA pellet with 5 ml of 75% ethanol. Centrifuge at ≥3,000 x g for 5 minutes (preferably at 15,000 x g for 10 minutes) at 4ºC. Carefully remove the supernatant then air-dry the DNA pellet for 10 minutes. Once the DNA pellet is dry, add 500 µl–2 ml (or a suitable volume) of TE¹ or water² then place the tube in a 60ºC 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.

¹Using 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.

²If using water, ensure the water pH is ≥8.0. ddH₂O should be fresh as ambient CO₂ can quickly cause acidification.
Geneaid™ Midi Plasmid Kit (Endotoxin Free) Protocol

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

IMPORTANT BEFORE USE!

1. For PIE25 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 PIE02 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 ddH2O

Protocol Procedure With Color Indicator

1. Harvesting

Transfer cultured bacterial cells to a 50 ml centrifuge tube then centrifuge at ≥3,000 x 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 up to 200 ml of high-copy or 100-300 ml of low-copy cultured bacterial cells using the same 50 ml centrifuge tube.

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

2. Equilibration

During centrifugation, place a Plasmid Midi Column in a new 50 ml centrifuge tube. Equilibrate the Plasmid Midi Column by adding 5 ml of PEQ Buffer. Allow the column to empty completely by gravity flow. Discard the flow-through and place the Plasmid Midi Column back in the 50 ml centrifuge tube then set it aside for Step 7.

3. Resuspension

Add 4 ml of PM1 Buffer (make sure RNase A was added) and 40 µl of TrueBlue Lysis Buffer to a new 50 ml centrifuge tube. Mix by shaking gently.

NOTE: It is normal for precipitates to form after mixing TrueBlue Lysis Buffer with PM1 Buffer.

Transfer the mixture to the 50 ml centrifuge tube 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.
4. Cell Lysis

Add **4 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 CO₂ 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:** 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.  

**Insufficient Mixing**  
**Correct Mixing**

5. Neutralization

Add **4 ml of PM3 Buffer** and mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Centrifuge at ≥3,000 x g for 20 minutes at room temperature.

**NOTE:** 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.  

**Insufficient Mixing**  
**Correct Mixing**
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 \textbf{1.2 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, \textit{transfer the mixture} to the equilibrated \textbf{Plasmid Midi Column}. Allow the column to empty completely by gravity flow. Discard the flow-through then place the \textbf{Plasmid Midi Column} back in the 50 ml centrifuge tube.

8. Wash

Wash the \textbf{Plasmid Midi Column} by adding \textbf{12 ml of PW Buffer} and allow the column to empty completely by gravity flow then discard the flow-through.

9. Elution

Place the \textbf{Plasmid Midi Column} in a clean 50 ml centrifuge tube then add \textbf{8 ml of PEL Buffer} to elute the DNA by gravity flow. Discard the \textbf{Plasmid Midi Column} once it has emptied completely.

10. DNA Precipitation

Add \textbf{6 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°C. Carefully remove the supernatant then wash the DNA pellet with \textbf{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°C. Carefully remove the supernatant then air-dry the DNA pellet for 10 minutes. Once the DNA pellet is dry, add \textbf{500 µl–2 ml (or a suitable volume) of TE\textsuperscript{1} or water\textsuperscript{2}} then place the tube in a 60°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.

\textsuperscript{1}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.

\textsuperscript{2}If using water, ensure the water pH is \(\geq 8.0\). ddH\textsubscript{2}O should be fresh as ambient CO\textsubscript{2} can quickly cause acidification.
## Protocol Procedure Without Color Indicator

### 1. Harvesting

Transfer **cultured bacterial cells** to a 50 ml centrifuge tube then centrifuge at ≥3,000 x 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 up to 200 ml of high-copy or 100-300 ml of low-copy cultured bacterial cells using the same 50 ml centrifuge tube.

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

### 2. Equilibration

During centrifugation, place a **Plasmid Midi Column** in a new 50 ml centrifuge tube. Equilibrate the **Plasmid Midi Column** by adding 5 ml of PEQ Buffer. Allow the column to empty completely by gravity flow. Discard the flow-through and place the **Plasmid Midi Column** back in the 50 ml centrifuge tube then set it aside for Step 7.

### 3. Resuspension

Add 4 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.

### 4. Cell Lysis

Add 4 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 CO₂ 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.
5. Neutralization

Add **4 ml of PM3 Buffer** and mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Centrifuge at ≥3,000 x g for 20 minutes at room temperature.

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

8. Wash

Wash the **Plasmid Midi Column** by adding **12 ml of PW Buffer** and allow the column to empty completely by gravity flow then discard the flow-through.

9. Elution

Place the **Plasmid Midi Column** in a clean 50 ml centrifuge tube then add **8 ml of PEL Buffer** to elute the DNA by gravity flow. Discard the **Plasmid Midi Column** once it has emptied completely.

10. DNA Precipitation

Add **6 ml (0.75 volumes) of isopropanol** to the eluted DNA from Step 9. Mix the tube completely by inverting then centrifuge at ≥3,000 x g for 20 minutes (preferably at 15,000 x g for 30 minutes) at 4°C. Carefully remove the supernatant then wash the DNA pellet with **5 ml of 75% ethanol.** Centrifuge at ≥3,000 x g for 5 minutes (preferably at 15,000 x g for 10 minutes) at 4°C. Carefully remove the supernatant then air-dry the DNA pellet for 10 minutes. Once the DNA pellet is dry, add **500 µl–2 ml (or a suitable volume) of TE¹ or water²** then place the tube in a 60°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.

¹Using 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.
²If using water, ensure the water pH is ≥8.0. ddH₂O should be fresh as ambient CO₂ can quickly cause acidification.
Troubleshooting

Low Yield

Incomplete buffer preparation.

For PI025, PIE25 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 PI002 and PIE02 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.

We recommend using a single freshly isolated *E. coli* colony to inoculate into 50-100 ml of LB medium. Solid and liquid medium should contain antibiotics. Do not use overgrown bacterial cultures (≤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 ≥8.0. ddH₂O should be fresh as ambient CO₂ can quickly cause acidification.
No yield of plasmid DNA.

Increase volume of low-copy number plasmid to 300 ml. We recommend using a single freshly isolated *E. coli* colony to inoculate into 50-100 ml of LB medium. Solid and liquid medium should contain antibiotics. Do not use overgrown bacterial cultures.

**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 2-8ºC 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 genomic DNA shearing. After adding PM3 Buffer, mix thoroughly then incubate the lysate on ice for 10-15 minutes to enhance precipitation and reduce genomic DNA contamination.

**Geneaid™ Midi Plasmid Kit Functional Test Data**

**Figure 1.** Plasmid DNA from a 100 ml overnight *E. coli* (DH5α) culture, containing plasmid pBluescript (OD600 = 3.65) was purified using the Geneaid™ Midi Plasmid Kit. The purified supercoiled plasmid DNA was used in EcoRI digestion and analyzed by electrophoresis on a 1% agarose gel. M = Geneaid 1 Kb DNA Ladder

<table>
<thead>
<tr>
<th>Test</th>
<th>DNA Conc.</th>
<th>260/280</th>
<th>260/230</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>211.2 µg/ml</td>
<td>1.87</td>
<td>2.27</td>
<td>422.4 µg</td>
</tr>
<tr>
<td>2</td>
<td>216.6 µg/ml</td>
<td>1.87</td>
<td>2.27</td>
<td>433.2 µg</td>
</tr>
<tr>
<td>3</td>
<td>224.8 µg/ml</td>
<td>1.87</td>
<td>2.28</td>
<td>449.6 µg</td>
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