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Instruction Manual
Ver. 04.30.13
For Research Use Only

I-Blue Midi Plasmid Kit

IB47180 (2 Preparation Sample Kit)
IB47181 (25 Preparation Kit)

Advantages

Sample: 50-150 ml of cultured bacterial cells
Yield: up to 400 µg of pure transfection grade plasmid DNA from 100 ml of cultured bacterial cells
Format: anion-exhange resin column, gravity flow
Operation Time: within 80 minutes
Elution Volume: 500 µl-2ml
Kit Storage: dry at room temperature (15-25ºC) for up to 2 years, PM1 and RNase A mixture should be stored at 2-8ºC for up to 6 months

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Introduction

The I-Blue Midi Plasmid Kit uses pre-packed anion-exchange resin columns to purify plasmid DNA from 50-150 ml of cultured bacterial cells. I-Blue 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. Due to the high efficiency of the Midi Column, the entire procedure can be completed in 80 minutes without ultracentrifuges, HPLC or other toxic reagents. 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 I-Blue Midi Plasmid Kit is tested on a lot-to-lot basis, by isolating plasmid DNA from a 50 ml overnight *E. coli* (DH5α) culture, containing plasmid pBluescript (A600 > 2 U/ml). Following the purification process, a yield of more than 250 µg is obtained and the A260/A280 ratio is between 1.8-2.0. The purified plasmid (1 µg) is used in *Eco*RI digestion, and analyzed by electrophoresis.

Kit Components

Component	IB47180	IB47181
PM1 Buffer[1]	10 ml	110 ml
PM2 Buffer[2]	10 ml	110 ml
PM3 Buffer	10 ml	110 ml
I-Blue Lysis Buffer	150 µl	1.5 ml
PEQ Buffer	12 ml	130 ml
PW Buffer	30 ml	360 ml
PEL Buffer	25 ml	220 ml
RNase A (50 mg/ml)	Added	200 µl
Midi Columns	2	25
Plasmid Midi Columns	2	25

[1]For IB47181 add provided RNase A to PM1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. PM1 and RNase A mixture should be stored at 2-8ºC for up to 6 months. For IB47180 samples, RNase A was already added to PM1.

[2]If precipitates have formed in PM2 Buffer, warm the buffer in a 37ºC 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)

The Midi Column is used with centrifugation to filter the neutralized colorless mixture and remove contaminants to facilitate DNA binding to the silica resin of the Plasmid Midi Column

DNA binding to silica resin while contaminants remain suspended

Wash (removal of contaminants while DNA remains bound to membrane)

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

I-Blue Midi Plasmid Kit Protocol

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

IMPORTANT BEFORE USE!

1. For IB47181 add provided RNase A to PM1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. PM1 and RNase A mixture should be stored at 2-8ºC for up to 6 months. For IB47180 samples, RNase A was already added to PM1.

2. If precipitates have formed in PM2 Buffer, warm the buffer in a 37ºC water bath followed by gentle shaking to dissolve.

Additional Requirements

50 ml centrifuge tubes, isopropanol, 70% ethanol, TE or ddH_20

Protocol Procedure With Color Indicator

1. Harvesting

Transfer **50-100 ml of high-copy plasmid or 100-150 ml of low-copy plasmid cultured bacterial cells** to a 50 ml centrifuge tube then centrifuge at 6,000 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 samples >50 ml 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 37ºC 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 (white membrane)** 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)** to a new 50 ml centrifuge tube. **Add 40 µl of I-Blue Lysis Buffer** to the same 50 ml centrifuge tube then mix by shaking gently.

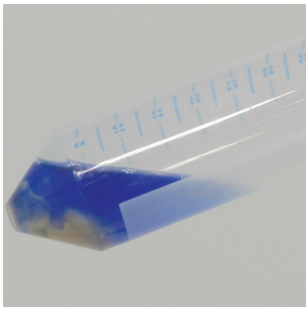
NOTE: It is normal for precipitates to form after mixing TrueBlue Lysis Buffer with PM1 Buffer.

Transfer the mixture to the 50 ml centrifuge tube containing the cell pellet. Resuspend the cell pellet completely by vortex or pipette. Continue to vortex or pipette until all traces of the cell pellet have been 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 CO_2 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.



Insufficient Mixing

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

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.

NOTE: After adding PM3 Buffer, the suspension will become colorless. If blue regions remain in the suspension, continue mixing until it becomes colorless.

Insufficient Mixing

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

Correct Mixing

6. DNA Binding

Place a **Midi Column (red membrane)** in a new 50 ml centrifuge tube then transfer all of the colorless suspension to the **Midi Column**. Centrifuge at 3,000 x g for 2 minutes at room temperature. Discard the **Midi Column** then transfer the flow-through to the equilibrated **Plasmid Midi Column (white membrane)**. 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 volume) of isopropanol** to the 50 ml centrifuge tube containing the eluted DNA from Step 8. Mix the tube completely by inverting then centrifuge at 15,000 x g for 15-20 minutes at 4ºC. Carefully remove the supernatant then wash the DNA pellet with **5 ml of 75% ethanol**. Centrifuge 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 2 ml (or a suitable volume) of TE[1] or water[2] 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.

[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.

[2]If using water, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification.

IMPORTANT BEFORE USE!

1. For IB47181 add provided RNase A to PM1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. PM1 and RNase A mixture should be stored at 2-8ºC for up to 6 months. For IB47180 samples, RNase A was already added to PM1.

2. If precipitates have formed in PM2 Buffer, warm the buffer in a 37ºC water bath followed by gentle shaking to dissolve.

Additional Requirements

50 ml centrifuge tubes, isopropanol, 70% ethanol, TE or ddH_20

Protocol Procedure Without Color Indicator

1. Harvesting

Transfer **50-100 ml of high-copy plasmid or 100-150 ml of low-copy plasmid cultured bacterial cells** to a 50 ml centrifuge tube then centrifuge at 6,000 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 samples >50 ml 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 37ºC 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 (white membrane)** 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)** to the 50 ml centrifuge tube containing the cell pellet. Resuspend the cell pellet completely by vortex or pipette. Continue to vortex or pipette until all traces of the cell pellet have been 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 CO_2 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.

6. DNA Binding

Place a **Midi Column (red membrane)** in a new 50 ml centrifuge tube then transfer all of the colorless suspension to the **Midi Column**. Centrifuge at 3,000 x g for 2 minutes at room temperature. Discard the **Midi Column** then transfer the flow-through to the equilibrated **Plasmid Midi Column (white membrane)**. 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 volume) of isopropanol** to the 50 ml centrifuge tube containing the eluted DNA from Step 8. Mix the tube completely by inverting then centrifuge at 15,000 x g for 15-20 minutes at 4ºC. Carefully remove the supernatant then wash the DNA pellet with **5 ml of 75% ethanol**. Centrifuge 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 2 ml (or a suitable volume) of TE[1] or water[2] 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.

[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.

[2]If using water, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification.

Troubleshooting

Low Yield

Incomplete buffer preparation.

For IB47181 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. For IB47180 samples, RNase A was already added to PM1. If precipitates have formed in PM2 Buffer, warm in a 37ºC water bath followed by gentle shaking to dissolve. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

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 37ºC with 150-180 rpm shaking).

Culture growth medium was not removed completely.

Following centrifugation in the Harvesting step, use a narrow pipet 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 I-Blue 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 I-Blue 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 Rehyrdation.

If using water to dissolve the DNA pellet, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification.

No yield of plasmid DNA.

Increase volume of low-copy number plasmid to 150 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.

I-Blue Midi Plasmid Kit Functional Test Data

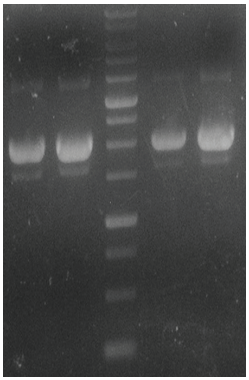


Figure 1. Plasmid DNA was extracted using both the I-Blue Midi Plasmid Kit (lane 1, 2) and the equivalent competitor's plasmid midi kit (lane 3, 4). The purified supercoiled plasmid DNA [50 ml and 100 ml overnight *E. coli* (DH5α) culture, containing a 3 kb plasmid pBluescript (A600 > 2 U/ml, OD600 = 3.8)], was used in *Eco*RI digestion and analyzed by electrophoresis on a 1% agarose gel. M = 1 Kb DNA Ladder, Lane 1: I-Blue Midi Plasmid Kit (50 ml), Lane 2: I-Blue Midi Plasmid Kit (100 ml), Lane 3: Equivalent Competitor Kit (50 ml), Lane 4: Equivalent Competitor Kit (100 ml)

Brand	Test Volume	260/280	260/230	Yield
IBI	50 ml	1.85	2.25	273.0 µg
	100 ml	1.87	2.14	409.2 µg
MN	50 ml	1.85	2.26	121.1 µg
	100 ml	1.87	2.33	289.8 µg

Related DNA/RNA Extraction Products

Plasmid DNA Purification		
Product	Package Size	Catalogue Number
I-Blue Mini Plasmid Kit	100/300 preps	IB47171/172
I-Blue Midi Plasmid Kit	25 preps	IB47181
I-Blue Midi Plasmid Kit (Endotoxin Free)	25 preps	IB47191
Fast Ion Plasmid Midi Kit	25 preps	IB47111
Fast Ion Plasmid Midi Kit (Endotoxin Free)	25 preps	IB47113
Fast Ion Plasmid Maxi Kit	10/25 preps	IB47121/122
Fast Ion Plasmid Maxi Kit (Endotoxin Free)	10/25 preps	IB47124/125
96-Well Plasmid Kit	4/10 x 96 preps	IB47151/152
Post Reaction DNA Purification		
Product	Package Size	Catalogue Number
Gel/PCR DNA Fragments Extraction Kit	100/300 preps	IB47020/030
Small DNA Fragments Extraction Kit	100/300 preps	IB47061/062
96-Well Gel/PCR DNA Extraction Kit	4/10 x 96 preps	IB47040/050
Genomic DNA Purification		
Product	Package Size	Catalogue Number
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	IB47201/202
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10 preps	IB47210
Genomic DNA Mini Kit (Tissue)	50/300 preps	IB47221/222
gMax Mini Kit (Blood/Tissue)	100/300 preps	IB47281/282
Genomic DNA Mini Kit (Plant)	100 preps	IB47230
Genomic DNA Maxi Kit (Plant)	10/25 preps	IB47240/241
gBAC Mini DNA Bacteria Kit	100/300 preps	IB47291/292
96-Well Genomic DNA Extraction Kit	4/10 x 96 preps	IB47251/252
96-Well Genomic DNA Extraction Kit (Plant)	4/10 x 96 preps	IB47271/272
RNA Purification		
Product	Package Size	Catalogue Number
Total RNA Mini Kit (Blood/Cultured Cell)	50/100/300 preps	IB47321/322/323
Total RNA Maxi Kit (Blood/Cultured Cell)	10 preps	IB47330
Total RNA Mini Kit (Tissue)	50/100 preps	IB47301/302
Total RNA Maxi Kit (Tissue)	10 preps	IB47310
Total RNA Mini Kit (Plant)	50/100 preps	IB47341/342
Total RNA Maxi Kit (Plant)	10 preps	IB47350
rBAC Mini RNA Bacteria Kit	100/300 preps	IB47421/412
rYeast Total RNA Mini Kit	50/100/300 preps	IB47411/422
96-Well Total RNA Extraction Kit (Plant)	4/10 x 96 preps	IB47381/382
96-Well Total RNA Extraction Kit	4/10 x 96 preps	IB47360/361
miRNA Isolation Kit	100 preps	IB47371
Virus DNA/RNA Purification		
Product	Package Size	Catalogue Number
Viral Nucleic Acid Extraction Kit II	50/100/300 preps	IB47401/402/403

For additional product information please visit www.ibisci.com. Thank you!

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