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Instruction Manual
Ver. 03.06.17
For Research Use Only

I-Blue Mini Plasmid Kit

IB47170 (4 Preparation Sample Kit)
IB47171 (100 Preparation Kit)
IB47172 (300 Preparation Kit)

Advantages

Sample: 1-7 ml of cultured bacterial cells
Yield: up to 50 µg of pure plasmid DNA
Format: plasmid spin column
Operation Time: within 15 minutes
Elution Volume: 30-100 µl
Kit Storage: dry at room temperature (15-25°C)

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Introduction

The I-Blue Mini Plasmid Kit was designed for rapid isolation of plasmid DNA from 1-7 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 and RNA contaminants. Typical yields are 20-35 µg for high-copy number plasmid or 3-10 µg for low-copy number plasmid from 4 ml of cultured bacterial cells. DNA phenol extraction or alcohol precipitation is not required and the entire procedure can be completed within 15 minutes. The purified plasmid DNA is ready for use in restriction enzyme digestion, ligation, PCR, and sequencing reactions.

Quality Control

The quality of the I-Blue Mini Plasmid Kit is tested on a lot-to-lot basis by isolating plasmid DNA from a 4 ml overnight *E. coli* (DH5α) culture containing plasmid pBluescript (A600 > 2 U/ml). Following the purification process, a yield of more than 20 µg is obtained and the A260/A280 ratio is between 1.8-2.0. The purified plasmid DNA (1 µg) is used in *Eco*RI digestion, and analyzed by electrophoresis.

Kit Components

Component	IB47170	IB47171	IB47172
PD1 Buffer[1]	1 ml	25 ml	65 ml
PD2 Buffer[2]	1 ml	25 ml	75 ml
PD3 Buffer	1.5 ml	45 ml	100 ml
I-Blue Lysis Buffer	10 µl	250 µl	650 µl
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer[3] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	1 ml	6 ml	30 ml
RNase A (50 mg/ml)	Added	100 µl	260 µl
PDH Columns	4	100	300
2 ml Collection Tubes	4	100	300

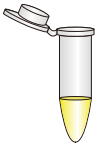
[1]For IB47171 and IB47172 add provided RNase A to PD1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. PD1 and RNase A mixture should be stored at 2-8ºC for up to 6 months. For IB47170 samples, RNase A was already added to PD1.

[2]If precipitates have formed in PD2 Buffer, warm the buffer in a 37ºC water bath, followed by gentle shaking to dissolve.

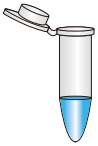
[3]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

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

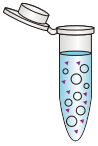
Quick Protocol Diagram



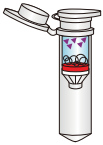
Harvest cultured bacterial cells by centrifuge to form a cell pellet, followed by resuspension



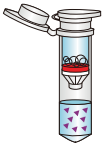
Lyse bacterial cells (optional color indicator will turn blue when lysis is successful)



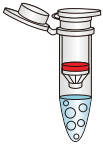
Neutralize suspension (optional color indicator will become clear when neutralization is successful)



DNA binding to membrane while contaminants remain suspended



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



Elution of pure plasmid DNA which is ready for subsequent reactions

I-Blue Mini Plasmid Kit Protocol

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

IMPORTANT BEFORE USE!

1. For IB47171 and IB47172 add provided RNase A to PD1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. PD1 and RNase A mixture should be stored at 2-8ºC for up to 6 months. For IB47170 samples, RNase A was already added to PD1.

2. If precipitates have formed in PD2 Buffer, warm the buffer in a 37ºC water bath followed by gentle shaking to dissolve.

3. 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.

Additional Requirements

1.5 ml microcentrifuge tubes, absolute ethanol

Protocol Procedure With Color Indicator

1. Harvesting

Transfer **1.5 ml of cultured bacterial cells** (1-2 x 10^9 *E. coli* grown in LB medium) to a 1.5 ml microcentrifuge tube. Centrifuge at 14-16,000 x g for 1 minute at room temperature to form a cell pellet then discard the supernatant completely. Use a narrow pipette tip to ensure the supernatant is completely removed. Repeat the Harvesting step as required for samples between 1.5-7.0 ml using the same 1.5 ml microcentrifuge tube.

NOTE: Using 2 OD600 - 6 OD600 units of bacterial culture is recommended. Do not use overgrown bacterial cultures (≤16 hours incubated in a culture tube 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. Resuspension

Add **200 µl of PD1 Buffer (make sure RNase A was added)** to a new 1.5 ml microcentrifuge tube. **Add 2 µl of I-Blue Lysis Buffer** to the same 1.5 ml microcentrifuge tube then mix by shaking gently.

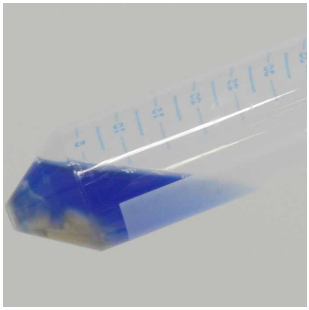
NOTE: It is normal for precipitates to form after mixing I-Blue with PD1 Buffer.

Transfer the mixture to the 1.5 ml microcentrifuge 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.

3. Cell Lysis

Add **200 µl of PD2 Buffer** to the resuspended sample then mix gently by inverting the tube 10 times. Close PD2 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 PD2 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

4. Neutralization

Add **300 µl of PD3 Buffer** and mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Centrifuge at 14-16,000 x g for 3 minutes at room temperature. If using >5 ml of bacterial cells, centrifuge at 16-20,000 x g for 5-8 minutes. During centrifugation, place a **PDH Column** in a 2 ml Collection Tube.

NOTE: After adding PD3 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

5. DNA Binding

Transfer all of the supernatant to the **PDH Column**. Use a narrow pipette tip to ensure the supernatant is completely transferred without disrupting the white precipitate. Centrifuge at 14-16,000 x g for 30 seconds at room temperature then discard the flow-through. Place the **PDH Column** back in the 2 ml Collection Tube.

6. Wash

For Improved Downstream Sequencing Reactions

Add **400 µl of W1 Buffer** into the **PDH Column**. Centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through then place the **PDH Column** back in the 2 ml Collection Tube. Proceed with Wash Buffer addition.

NOTE: W1 Buffer is essential for efficient sequencing reactions by removing nuclease contamination and should be added prior to Wash Buffer addition. If you are not performing sequencing reactions, W1 Buffer is not required. Proceed directly to Wash Buffer addition.

For Standard Plasmid DNA Purification

Add **600 µl of Wash Buffer (make sure absolute ethanol was added)** into the **PDH Column**. Centrifuge at 14-16,000 x g for 30 seconds at room temperature. Discard the flow through then place the **PDH Column** back in the 2 ml Collection Tube. Centrifuge at 14-16,000 x g for 3 minutes at room temperature to dry the column matrix. Transfer the dried **PDH Column** to a new 1.5 ml microcentrifuge tube.

NOTE: Perform Wash Buffer steps twice for salt sensitive downstream applications.

7. Elution

Add **50 µl of Elution Buffer**[1], TE[2] or water[3] into the **CENTER** of the column matrix. Let stand for at least 2 minutes to allow Elution Buffer, TE or water to be completely absorbed. Centrifuge at 14-16,000 x g for 2 minutes at room temperature to elute the purified DNA.

[1]If a higher DNA concentration is required, use 30 µl of Elution Buffer (10 mM Tris-HCl, pH8.5) then repeat the Elution step by adding the same 30 µl of Elution Buffer (which now contains the eluted DNA) to the center of the column matrix again. If maximum DNA yield is required, use 100 µl of Elution Buffer (DNA concentration will be diluted). Ensure that Elution Buffer is added into the center of the PDH Column matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated Elution Buffer (60~70ºC).

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the PDH Column matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated TE (60~70ºC).

[3]If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the PDH Column matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated water (60~70ºC).

IMPORTANT BEFORE USE!

1. For IB47171 and IB47172 add provided RNase A to PD1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. PD1 and RNase A mixture should be stored at 4ºC for up to 6 months. For IB47170 samples, RNase A was already added to PD1.

2. If precipitates have formed in PD2 Buffer, warm the buffer in a 37ºC water bath followed by gentle shaking to dissolve.

3. 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.

Additional Requirements

1.5 ml microcentrifuge tubes, absolute ethanol

Protocol Procedure Without Color Indicator

1. Harvesting

Transfer **1.5 ml of cultured bacterial cells** (1-2 x 10^9 *E. coli* grown in LB medium) to a 1.5 ml microcentrifuge tube. Centrifuge at 14-16,000 x g for 1 minute at room temperature to form a cell pellet then discard the supernatant completely. Use a narrow pipette tip to ensure the supernatant is completely removed. Repeat the Harvesting step as required for samples between 1.5-7.0 ml using the same 1.5 ml microcentrifuge tube.

NOTE: Using 2 OD600 - 6 OD600 units of bacterial culture is recommended. Do not use overgrown bacterial cultures (≤16 hours incubated in a culture tube 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. Resuspension

Add **200 µl of PD1 Buffer (make sure RNase A was added)** to the 1.5 ml microcentrifuge 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.

3. Cell Lysis

Add **200 µl of PD2 Buffer** to the resuspended sample then mix gently by inverting the tube 10 times. Close PD2 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.

4. Neutralization

Add **300 µl of PD3 Buffer** and mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Centrifuge at 14-16,000 x g for 3 minutes at room temperature. If using >5 ml of bacterial cells, centrifuge at 16-20,000 x g for 5-8 minutes. During centrifugation, place a **PDH Column** in a 2 ml Collection Tube.

5. DNA Binding

Transfer all of the supernatant to the **PDH Column**. Use a narrow pipette tip to ensure the supernatant is completely transferred without disrupting the white precipitate. Centrifuge at 14-16,000 x g for 30 seconds at room temperature then discard the flow-through. Place the **PDH Column** back in the 2 ml Collection Tube.

6. Wash

For Improved Downstream Sequencing Reactions

Add **400 µl of W1 Buffer** into the **PDH Column**. Centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through then place the **PDH Column** back in the 2 ml Collection Tube. Proceed with Wash Buffer addition.

NOTE: W1 Buffer is essential for efficient sequencing reactions by removing nuclease contamination and should be added prior to Wash Buffer addition. If you are not performing sequencing reactions, W1 Buffer is not required. Proceed directly to Wash Buffer addition.

For Standard Plasmid DNA Purification

Add **600 µl of Wash Buffer (make sure absolute ethanol was added)** into the **PDH Column**. Centrifuge at 14-16,000 x g for 30 seconds at room temperature. Discard the flow through then place the **PDH Column** back in the 2 ml Collection Tube. Centrifuge at 14-16,000 x g for 3 minutes at room temperature to dry the column matrix. Transfer the dried **PDH Column** to a new 1.5 ml microcentrifuge tube.

NOTE: Perform Wash Buffer steps twice for salt sensitive downstream applications.

7. Elution

Add **50 µl of Elution Buffer**[1], TE[2] or water[3] into the **CENTER** of the column matrix. Let stand for at least 2 minutes to allow Elution Buffer, TE or water to be completely absorbed. Centrifuge at 14-16,000 x g for 2 minutes at room temperature to elute the purified DNA.

[1]If a higher DNA concentration is required, use 30 µl of Elution Buffer (10 mM Tris-HCl, pH8.5) then repeat the Elution step by adding the same 30 µl of Elution Buffer (which now contains the eluted DNA) to the center of the column matrix again. If maximum DNA yield is required, use 100 µl of Elution Buffer (DNA concentration will be diluted). Ensure that Elution Buffer is added into the center of the PDH Column matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated Elution Buffer (60~70ºC).

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the PDH Column matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated TE (60~70ºC).

[3]If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the PDH Column matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated water (60~70ºC).

Troubleshooting

Low Yield

Incomplete buffer preparation.

For IB47171 and IBI47172 add provided RNase A to PD1 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 IBI47170, RNase A was already added to PD1. If precipitates have formed in PD2 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 1-10 ml of LB medium. Solid and liquid medium should contain antibiotics. Do not use overgrown bacterial cultures (≤16 hours incubated in a culture tube at 37ºC 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. Continue to vortex or pipette until all traces of the cell pellet have been dissolved.

Bacterial cells were not lysed completely.

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

When using I-Blue Lysis Buffer: Following PD2 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 PD3 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 Elution step.

Ensure that Elution Buffer, TE or water is added into the center of the PDH Column matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated Elution Buffer, TE, or water (60~70ºC). If using water for elution, 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 5-7 ml. We recommend using a single freshly isolated *E. coli* colony to inoculate into 1-10 ml of LB medium. Solid and liquid medium should contain antibiotics. Do not use overgrown bacterial cultures. Use fresh cultures only.

Eluted DNA Does Not Perform Well In Downstream Applications

Residual ethanol contamination.

Following the Wash Step, dry the PDH Column with additional centrifugation at 14-16,000 x g for 5 minutes.

Residual salt contamination.

Perform the Wash Step twice for salt sensitive downstream applications.

RNA contamination.

Add provided RNase A to PD1 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. After adding PD2 Buffer to the sample mixture, mix gently by inverting the tube 10 times then let stand at room temperature for 2-5 minutes.

Genomic DNA contamination.

Do not use overgrown bacterial cultures. Use only fresh cultures as they will contain less genomic DNA than old cultures. During PD2 and PD3 Buffer addition, mix gently to prevent genomic DNA shearing.

Bacterial cells were not neutralized completely.

When using I-Blue Lysis Buffer: Following PD3 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.

Nuclease contamination.

Following the DNA Binding step, add 400 µl of W1 Buffer into the PDH Column. Centrifuge the PDH Column at 14-16,000 x g for 30 seconds at room temperature then proceed with Wash Buffer addition.

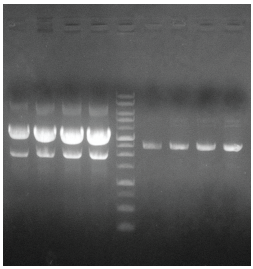
I-Blue Mini Plasmid Kit Functional Test Data

Plasmid DNA Yield Based on Cell Culture Volume and *E. coli* Strain

E. coli Strain	Host Strain Cell Culture Volume (OD600 = 4.0)			
	1.5 ml	3 ml	5 ml	7 ml
DH5α	13-15 µg	27-29 µg	36-38 µg	40-42 µg
TOP10	10-12 µg	18-20 µg	25-27 µg	27-29 µg
BL21(DE3)	7-9 µg	13-15 µg	17-19 µg	20-22 µg

Table 1. Plasmid DNA was extracted using the I-Blue Mini Plasmid Kit. 10 µl aliquats of a 100 µl eluate of purified super coiled plasmid DNA from 1.5, 3, 5 and 7 ml overnight *E. coli* DH5α, TOP10 and BL21(DE3) cultures, containing a 3 kb plasmid pBluescript (OD600 = 4 U/ml) were analyzed by spectrophotometer.

Plasmid DNA Yield Based on Copy-Number



Copy Number	Host Strain Cell Culture Volume (OD600 = 4.0)			
	1.5 ml	3 ml	5 ml	7 ml
High-Copy (pBluescript)	13-15 µg	27-29 µg	36-38 µg	40-42 µg
Low-Copy (pBR322)	4-6 µg	8-10 µg	12-14 µg	18-20 µg

Table 2. Yield of purified plasmid DNA (100 µl eluate) from 1.5, 3, 5 and 7 ml of cultured bacterial cells including high-copy number plasmid pBluescript and low-copy number plasmid pBR322.

Figure 1. Plasmid DNA was extracted using the I-Blue Mini Plasmid Kit. 5 µl aliquats of a 100 µl eluate of purified super coiled plasmid DNA from 1.5, 3, 5 and 7 ml overnight *E. coli* (DH5α) culture, containing a 3 kb plasmid pBluescript and pBR322 (OD600 = 4 U/ml) were used in *Eco*RI digestion and analyzed by electrophoresis on a 0.8% agarose gel.

M = 1 Kb DNA Ladder
Lane 1: 1.5 ml bacterial culture containing pBluescript
Lane 2: 3 ml bacterial culture containing pBluescript
Lane 3: 5 ml bacterial culture containing pBluescript
Lane 4: 7 ml bacterial culture containing pBluescript
Lane 5: 1.5 ml bacterial culture containing pBR322
Lane 6: 3 ml bacterial culture containing pBR322
Lane 7: 5 ml bacterial culture containing pBR322
Lane 8: 7 ml bacterial culture containing pBR322

Plasmid DNA Yield Based on DNA Size

5 ml of DH5α Cell Culture (OD600 = 4.0)	
DNA Size	DNA Yield
3.0 kb	36-38 µg
5.7 kb	36-38 µg
7.7 kb	36-38 µg
13 kb (Low-Copy Number)	13-15 µg

Table 3. Yield of 3.0, 5.7, 7.7 and 13 kb purified plasmid DNA (100 µl eluate) from 5 ml of DH5α cultured bacterial cells.

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