For Research Use Only

IBI SCIENTIFIC

INSTRUCTION MANUAL

I-Blue Mini Plasmid Endo Free Kit

IB47175 (4 Preparation Sample Kit)

IB47176 (100 Preparation Kit)

Advantages

Sample:	1-5 ml of cultured E. coli cells
Yield:	up to 40 µg of transfection grade plasmid DNA
Endotoxin Removal:	< 0.1 EU/µg of plasmid DNA
Format:	plasmid spin column
Operation Time:	within 50 minutes
Elution Volume:	30-100 µl
Kit Storage:	dry at room temperature (15-25°C)

Introduction

The I-Blue Mini Plasmid Endo Free Kit was designed for transfection grade plasmid DNA purification from 1-5 ml of cultured E. coli cells using silica-membrane spin columns. A modified alkaline lysis method and RNase treatment are used to obtain clear cell lysate with minimal genomic DNA and RNA contaminants. In addition, an efficient endotoxin removal step is integrated in the procedure and endotoxins are precipitated in a proprietary reagent. 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. DNA phenol extraction or alcohol precipitation is not required and the entire procedure can be completed within 50 minutes. The purified endotoxin free plasmid DNA is ready for use in transfection, restriction enzyme digestion, ligation, PCR, and sequencing reactions.

Quality Control

The quality of the I-Blue Mini Plasmid Endo Free 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 25 µg is obtained and the A260/A280 ratio is between 1.8-2.0. The purified plasmid DNA (1 µg) is used in EcoRI digestion, and analyzed by electrophoresis.

Kit Contents

Component	IB47820	IB47821
PE1 Buffer[1]	1 ml	25 ml
PE2 Buffer[2]	1 ml	25 ml
PE3 Buffer	1 ml	25 ml
PE4 Buffer	0.5 ml	12.5 ml
PE5 Buffer	1.5 ml	40 ml
I-Blue Lysis Buffer	10 µl	250 µl
W1 Buffer	2 ml	45 ml
Wash Buffer[3] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)
Elution Buffer	1 ml	6 ml
RNase A (50 mg/ml)	Added	100 µl
PDH Columns	4	100
2 ml Collection Tubes	4	100

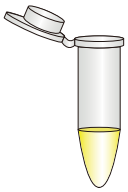
[1] For IB47176 add provided RNase A to PE1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. PE1 and RNase A mixture should be stored at 2-8°C for up to 6 months. For IB47175 samples, RNase A was already added to PE1.

[2] If precipitates have formed in PE2 Buffer, warm the buffer in a 37°C water bath, followed by gentle shaking to dissolve.

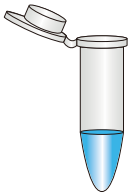
[3] Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

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

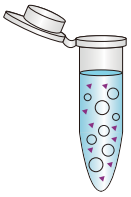
Quick Protocol Diagram



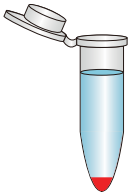
1. Harvest cultured bacterial cells by centrifuge to form a cell pellet, followed by resuspension



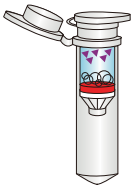
2. Lyse bacterial cells (optional color indicator will turn blue when lysis is successful)



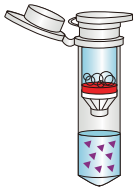
3. Neutralize suspension (optional color indicator will become clear when neutralization is successful)



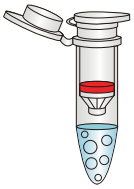
4. Endotoxin is precipitated in the red pellet followed by endotoxin removal.



5. Endotoxin free DNA binding to membrane while contaminants remain suspended



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



7. Elution of pure Endotoxin Free plasmid DNA which is ready for subsequent reactions

I-Blue Mini Plasmid Endo Free Kit Protocol

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

IMPORTANT BEFORE USE!

1. For IB47176 add provided RNase A to PE1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. PE1 and RNase A mixture should be stored at 2-8°C for up to 6 months. For IB47175 samples, RNase A was already added to PE1.
2. If precipitates have formed in PE2 Buffer, warm the buffer in a 37°C 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, ice, 65°C incubator

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 1.5-5.0 ml using the same 1.5 ml microcentrifuge tube.

NOTE: Using 2 OD600 – 4 OD600 units of bacterial culture is recommended. Do not use overgrown bacterial cultures (≤16 hours incubated in a culture tube 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. Resuspension

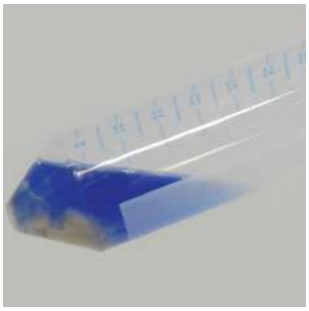
Add 200 µl of PE1 Buffer (make sure RNase A was added) and 2 µl of I-Blue Lysis Buffer to the 1.5 ml microcentrifuge tube containing the cell pellet. Resuspend the cell pellet completely by vortex or pipette. Continue to vortex or pipette until all traces of the cell pellet have been dissolved.

NOTE: It is normal for precipitates to form after mixing I-Blue Lysis Buffer with PE1 Buffer.

3. Cell Lysis

Add 200 µl of PE2 Buffer to the resuspended sample then mix gently by inverting the tube 10 times. Close PE2 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 PE2 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**

4. Neutralization

Add 200 µl of PE3 Buffer then 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 5 minutes at room temperature. Transfer all of the clear supernatant to a new 1.5 ml microcentrifuge tube without disrupting the white precipitate.

NOTE: After adding PE3 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

5. Endotoxin Removal

NOTE: If endotoxin free plasmid DNA is not required, please skip this step and proceed with step 6: DNA Binding.

Add 60 µl of PE4 Buffer (red endotoxin removal solution) to the lysate then vortex to mix.

NOTE: PE4 Buffer is a viscous solution. To pipette PE4 Buffer, draw up and dispense slowly. The solution will become turbid after the addition of PE4 Buffer. Incubate the sample mixture on ice for 5 minutes then heat at 65°C for 5 minutes. Centrifuge at 14-16,000 x g for 5 minutes.

NOTE: After centrifugation, the top aqueous phase will be clear and the bottom phase will be red and viscous.

Transfer the upper aqueous phase to a new 1.5 ml microcentrifuge tube. DO NOT contact the red bottom phase as this contains endotoxin. Repeat the above extraction procedure one more time. Transfer the upper aqueous phase to a new 1.5 ml microcentrifuge tube. Centrifuge the tube at 14-16,000 x g for 5 minutes. If a small red pellet is present at the bottom of the tube, transfer the supernatant to a new 1.5 ml microcentrifuge tube without disturbing the red pellet.

6. DNA Binding

Add 350 µl of PE5 Buffer to the sample and vortex to mix. Place a PDH Column in a 2 ml Collection Tube. Transfer 700 µl of the sample mixture to the PDH Column. 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. Transfer the remaining sample mixture to the PDH Column. 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.

7. 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.

8. 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 (60~70°C).

[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 (60~70°C).

[3] If using water for elution, ensure the water pH is ≥8.0. ddH2O should be fresh as ambient CO2 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 (60~70°C).

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 1.5-5.0 ml using the same 1.5 ml microcentrifuge tube.

NOTE: Using 2 OD600 – 4 OD600 units of bacterial culture is recommended. Do not use overgrown bacterial cultures (≤16 hours incubated in a culture tube 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. Resuspension

Add 200 µl of PE1 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 PE2 Buffer to the resuspended sample then mix gently by inverting the tube 10 times. Close PE2 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.

4. Neutralization

Add 200 µl of PE3 Buffer then 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 5 minutes at room temperature. Transfer all of the clear supernatant to a new 1.5 ml microcentrifuge tube without disrupting the white precipitate.

5. Endotoxin Removal

NOTE: If endotoxin free plasmid DNA is not required, please skip this step and proceed with step 6: DNA Binding.

Add 60 µl of PE4 Buffer (red endotoxin removal solution) to the lysate then vortex to mix.

NOTE: PE4 Buffer is a viscous solution. To pipette PE4 Buffer, draw up and dispense slowly. The solution will become turbid after the addition of PE4 Buffer. Incubate the sample mixture on ice for 5 minutes then heat at 65°C for 5 minutes. Centrifuge at 14-16,000 x g for 5 minutes.

NOTE: After centrifugation, the top aqueous phase will be clear and the bottom phase will be red and viscous.
Transfer the upper aqueous phase to a new 1.5 ml microcentrifuge tube. DO NOT contact the red bottom phase as this contains endotoxin. Repeat the above extraction procedure one more time. Transfer the upper aqueous phase to a new 1.5 ml microcentrifuge tube. Centrifuge the tube at 14-16,000 x g for 5 minutes. If a small red pellet is present at the bottom of the tube, transfer the supernatant to a new 1.5 ml microcentrifuge tube without disturbing the red pellet.

6. DNA Binding

Add 350 µl of PE5 Buffer to the sample and vortex to mix. Place a PDH Column in a 2 ml Collection Tube. Transfer 700 µl of the sample mixture to the PDH Column. 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. Transfer the remaining sample mixture to the PDH Column. 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.

7. 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.

8. 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 (60~70°C).

[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 (60~70°C).

[3] If using water for elution, ensure the water pH is ≥8.0. ddH2O should be fresh as ambient CO2 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 (60~70°C).

Troubleshooting

Low Yield

Incomplete buffer preparation.
For IB47176 add provided RNase A to PE1 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 IB47175, RNase A was already added to PE1. If precipitates have formed in PE2 Buffer, warm in a 37°C 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 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. Continue to vortex or pipette until all traces of the cell pellet have been dissolved.

Bacterial cells were not lysed completely.
Using 2 OD600 – 4 OD600 units of bacterial culture is recommended. When using I-Blue Lysis Buffer: Following PE2 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 PE3 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 (60~70°C). If using water for elution, 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 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 PE1 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. After adding PE2 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 PE2 and PE3 Buffer addition, mix gently to prevent genomic DNA shearing.

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.

Presto™ Mini Plasmid Kit Functional Test Data

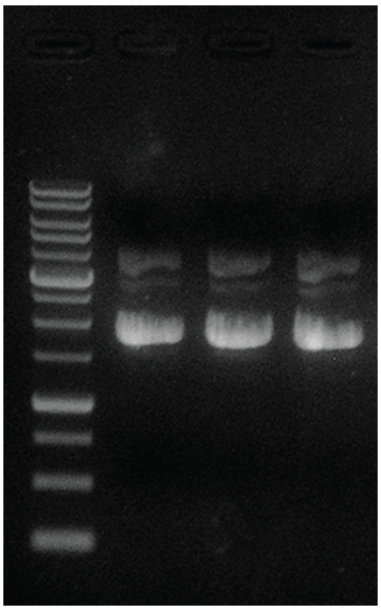


Figure 1.
Plasmid DNA was extracted using the I-Blue Mini Plasmid Endo Free Kit from a 4 ml of overnight cultured E. coli strain DH5α harboring pBluescript. 2 µl aliquots of a 50 µl eluate of purified super coiled plasmid were analyzed by spectrophotometer and 0.8% agarose gel.
M = 1 Kb DNA Ladder

Sample	ng/µl	260/280	260/230	Yield (ng)
1	569.4	1.89	2.32	28.5
2	578.4	1.89	2.30	28.9
3	594.4	1.89	2.31	29.7

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