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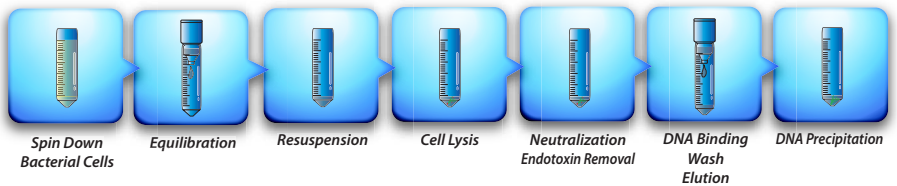
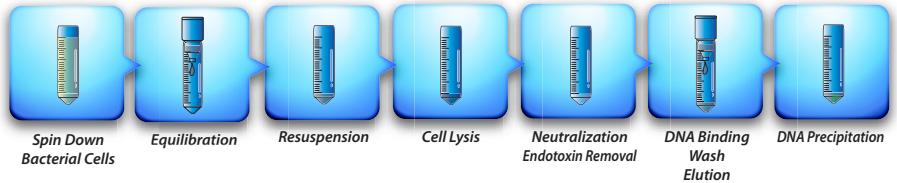
Real Genomics™

Store at room temperature 15°C~25°C

Workflow without using BlueMix Lysis Buffer



Workflow while using BlueMix Lysis Buffer



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Fastlon™ EndoFree Plasmid Midi Kit Protocol Book

Ultrapure Endotoxin-Free DNA from 50 -300 ml of Bacterial Cultures

Cat. No. **YPIF25**

Index

Real Genomics™

Fastlon™ EndoFree Plasmid Midi Kit

Fastlon™ EndoFree Plasmid Midi Kit

Store at room temperature 15°C~25°C

Cat. No. **YPIF25**
25 midi preps / kit
PM1 Buffer: 110 ml *
PM2 Buffer: 110 ml **
PM3 Buffer: 110 ml
BlueMix Lysis Buffer: 1.5 ml
PEQ Buffer: 130 ml
PER Buffer: 40 ml
PMC Buffer: 385 ml
PEL Buffer: 220 ml
RNase A: 200 µl
PI Column: 25 pcs

Sample: 50-300 ml of bacterial cultures (high-copy plasmid: 50-200 ml, low-copy plasmid: 100-300 ml)
Yield: 350-400 µg of endotoxin-free, pure transfection grade plasmid DNA (1-20 kb) from 100 ml of cultured bacterial cells
Format: Anion-exchange resin column, gravity flow
Operation Time: Within 110 minutes
Elution Volume: 500 µl-2 ml

Mix following buffers prior to the initial use:
* Add provided RNase A to PM1 Buffer and mix by shaking for a few seconds. Check the box on the bottle label showing RNase A is added. After addition of RNase A and optional BlueMix Lysis Buffer, PM1 Buffer is stable for 6 months when stored at 2–8°C.
** If precipitates have formed in PM2 Buffer, warm the buffer in a 37°C waterbath, followed by gentle shaking to dissolve before use.

Description
Fastlon™ EndoFree Plasmid Midi Kit is designed for rapid isolation of 350-400 µg of endotoxin-free, pure transfection grade plasmid DNA from 100 ml of cultured bacterial cells without ultracentrifuges, HPLC or other toxic reagents within 110 minutes. A modified alkaline lysis method and RNase treatment are used to obtain clear cell lysate with minimal genomic DNA/RNA contaminants. BlueMix Lysis Buffer (an optional color indicator) is also included in this kit to provide visual identification of optimum buffer mixing and prevent common handling errors. DNA purified with Fastlon™ EndoFree Plasmid Midi Kit contains only negligible amounts of endotoxin (<0.1 EU/µg plasmid DNA), verified by Limulus amoebocyte lysate (LAL). The ultrapure endotoxin-free plasmid DNA is ready for direct use in many sensitive downstream applications.

Features
Purification of 350-400 µg of endotoxin-free plasmid DNA from 100 ml of bacterial cultures within 110 minutes.
BlueMix Lysis Buffer provides visual identification of optimum buffer mixing and prevent common handling errors.
Phenol, chloroform or alcohol are not required.

Applications
Transfection grade plasmid DNA (Endotoxin Free) is ready for direct use in transfection, sequencing reactions, ligation, PCR, in-vitro transcription, microinjection, restriction enzyme digestion and gene gun.

Quality Control
The quality of Fastlon™ EndoFree Plasmid Midi Kits are tested on a lot-to-lot basis by isolation of plasmid DNA from 100 ml overnight culture of E.coli DH5a transformed with the plasmid pBluescript (A_{600} > 2 units/ml). Purified DNA is quantified with a spectrophotometer and the yield of plasmid DNA is more than 350 µg with A_{260}/A_{280} ratio 1.8 to 2.0. The purified DNA is used in EcoR1 digestion and checked by electrophoresis.

Storage
Fastlon™ EndoFree Plasmid Midi Kits should be stored dry at room temperature (15–25°C) for up to 2 years without showing any reduction in performance and quality. After addition of RNase A and optional BlueMix Lysis Buffer, PM1 Buffer is stable for 6 months when stored at 2–8°C. RNase A stock solution can be stored for 1 year at room temperature.

Important Notes
Please read the entire notes before starting any of the protocol procedures.

Growth of Bacterial Cultures
1. Using bacterial culture (O.D.600 of 2-6) is recommended.
2. Use fresh bacterial cultures only. Incubate for 12–16 hours at 37°C with 150-180 rpm shaking.
3. Do not use overgrown bacterial cultures. Growth for more than 16 hours is not recommended.
4. Solid medium and liquid medium (i.e. LB medium) should contain an antibiotic, such as ampicillin.

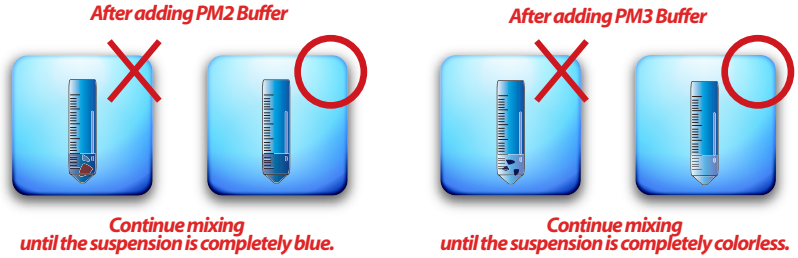
Buffer Notes
1. Before use, briefly centrifuge the provided RNase A and add entire RNase A to PM1 Buffer. Shake the mixture for a few seconds and store the mixture at 2–8°C. Check the box on the bottle label showing RNase A is added. After addition of RNase A and optional BlueMix Lysis Buffer, PM1 Buffer is stable for 6 months when stored at 2–8°C.
2. Check PM2 Buffer before use. Dissolve any precipitate by warming to 37°C, followed by gentle shaking.
3. Most buffers contain irritants. Always wear a lab coat, disposable gloves, and protective goggles when handling these buffers.
4. Optional: Add the provided BlueMix Lysis Buffer to PM1 Buffer (RNase A added) and mix before use. BlueMix Lysis Buffer should be added to PM1 Buffer at a ratio of 1:100 to achieve the optimal working concentration. e.g., 2 µl of BlueMix Lysis Buffer into 200 µl of PM1 Buffer. Using a simple color indicator, BlueMix Lysis Buffer prevents common handling errors, ensuring efficient cell lysis and neutralization.

Additional Requirements
1. 50 ml centrifuge tubes.
2. Isopropanol.
3. 75% ethanol.
4. TE or ddH20.

Recommended Culture Volumes
1. OD600 = 2, use 200 ml of high-copy number plasmid or 300 ml of low-copy number plasmid.
2. OD600 = 4, use 100 ml of high-copy number plasmid or 150 ml of low-copy number plasmid.

DNA Precipitation Notes
1. Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) to dissolve the DNA pellet is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications.
2. If using water to dissolve the DNA pellet, ensure its pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification.

How BlueMix Lysis Buffer Works
1. BlueMix Lysis Buffer precipitates after addition into PM1 Buffer.
2. Precipitates will be completely dissolved after addition of PM2 Buffer. The color of the suspension changes to blue. If colorless regions or brownish cell clumps are still visible, continue mixing until the suspension is completely blue.
3. After adding PM3 Buffer, the suspension turns colorless. If blue regions remain in the suspension, continue mixing until it becomes completely colorless. No traces of blue indicates correct mixing.

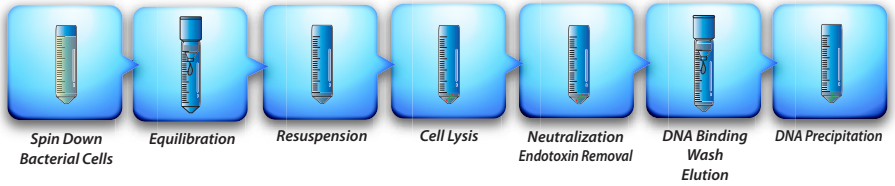


Protocol

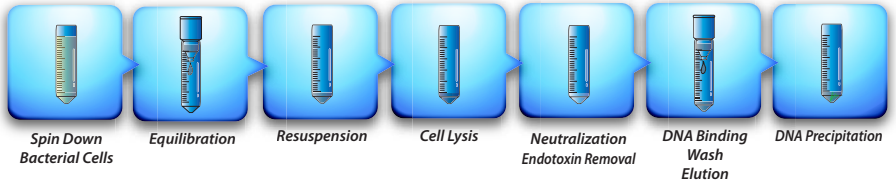
Please read the entire important notes before starting any of the protocol procedures.

This protocol is designed for rapid isolation of around 400 µg of endotoxin-free plasmid DNA from 100 ml of cultured bacterial cells without ultracentrifuges, HPLC or other toxic reagents. The entire procedure can be completed within 110 minutes.

Workflow without using BlueMix Lysis Buffer



Workflow while using BlueMix Lysis Buffer



Use of BlueMix Lysis Buffer is optional and is not required to successfully perform plasmid DNA extraction. BlueMix Lysis Buffer is an color indicator included in this kit to provide visual identification of optimum buffer mixing and prevent common handling errors.

Things to do before starting

1) Briefly centrifuge the provided RNase A and add entire RNase A to PM1 Buffer. Shake the mixture for a few seconds and store the mixture at 2–8°C. Check the box on the bottle label showing the RNase A is added. After addition of RNase A and optional BlueMix Lysis Buffer, PM1 Buffer is stable for 6 months when stored at 2–8°C.

2) Check PM2 Buffer before use. Dissolve any precipitate by warming to 37°C, followed by gentle shaking.

Harvesting the bacterial cells

1. Transfer 200 ml of high-copy plasmid (OD600 = 2) or 300 ml of low-copy plasmid (OD600 = 2) cultured bacterial cells to a 50 ml centrifuge tube and then centrifuge at ≥3,000 x g for 15 minutes 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 more than 50 ml by using the same 50 ml centrifuge tube.

Equilibration

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

Resuspension

Steps without using BlueMix Lysis Buffer

3. 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.

Steps while using BlueMix Lysis Buffer

3. Add 4 ml of PM1 Buffer (make sure RNase A was added) to a new 50 ml centrifuge tube. Add 40 µl of BlueMix Lysis Buffer to the same 50 ml centrifuge tube then mix by shaking gently. 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.

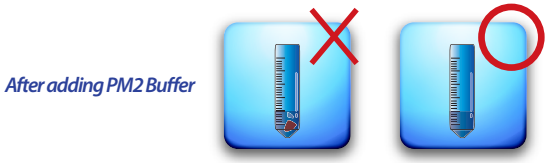
It is normal for precipitates to form after mixing BlueMix Lysis Buffer with PM1 Buffer. BlueMix Lysis Buffer should be added to PM1 Buffer at a ratio of 1:100 to achieve the optimal working concentration. e.g., 2 µl of BlueMix Lysis Buffer into 200 µl of PM1 Buffer.

Cell Lysis

4. Add 4 ml of PM2 Buffer to the resuspended sample then mix gently by inverting the tube 10 times. Do not vortex to avoid shearing genomic DNA. Close PM2 Buffer bottle immediately after use to avoid CO_2 acidification.
5. Let stand at room temperature for at least 2 minutes to ensure the lysate is homogeneous. Do not exceed 5 minutes.

Notes while using BlueMix Lysis Buffer

After adding PM2 Buffer, any precipitates will be completely dissolved and the color of the suspension will become blue. If colorless regions or brownish cell clumps are still visible, continue mixing until the suspension is completely blue.

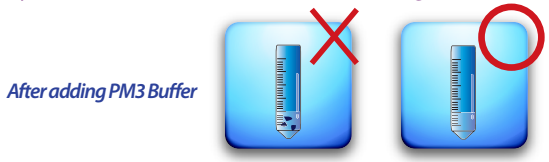


Neutralization

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

Notes while using BlueMix Lysis Buffer

After adding PD3 Buffer, the suspension turns colorless. If blue regions remain in the suspension, continue mixing until it becomes completely colorless. No traces of blue indicates correct mixing.



Endotoxin Removal

7. 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. Mix by inverting 5-10 times then incubate on ice for 30 minutes.

Following PER Buffer addition, the mixture will become cloudy.

DNA Binding

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

Wash

9. Wash the PI Column by adding 15 ml of PMC Buffer and allow the column to empty completely by gravity flow then discard the flow-through.

Elution

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

DNA Precipitation

11. Add 6 ml (0.75 volume) of isopropanol to the 50 ml centrifuge tube containing the eluted DNA from Elution Step. Mix the tube completely by inverting then centrifuge at 15,000 x g for 20~30 minutes at 4°C. Carefully remove the supernatant then wash the DNA pellet with 5 ml of 75% ethanol. Avoid contacting the DNA pellet while removing the supernatant.
12. Centrifuge at 15,000 x g for 5~10 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 TE[1] or water[2] then place the tube in a 60°C water bath for 5-10 minutes to dissolve the DNA pellet. Avoid contacting the DNA pellet while removing the supernatant.

[1] Using TE is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications.

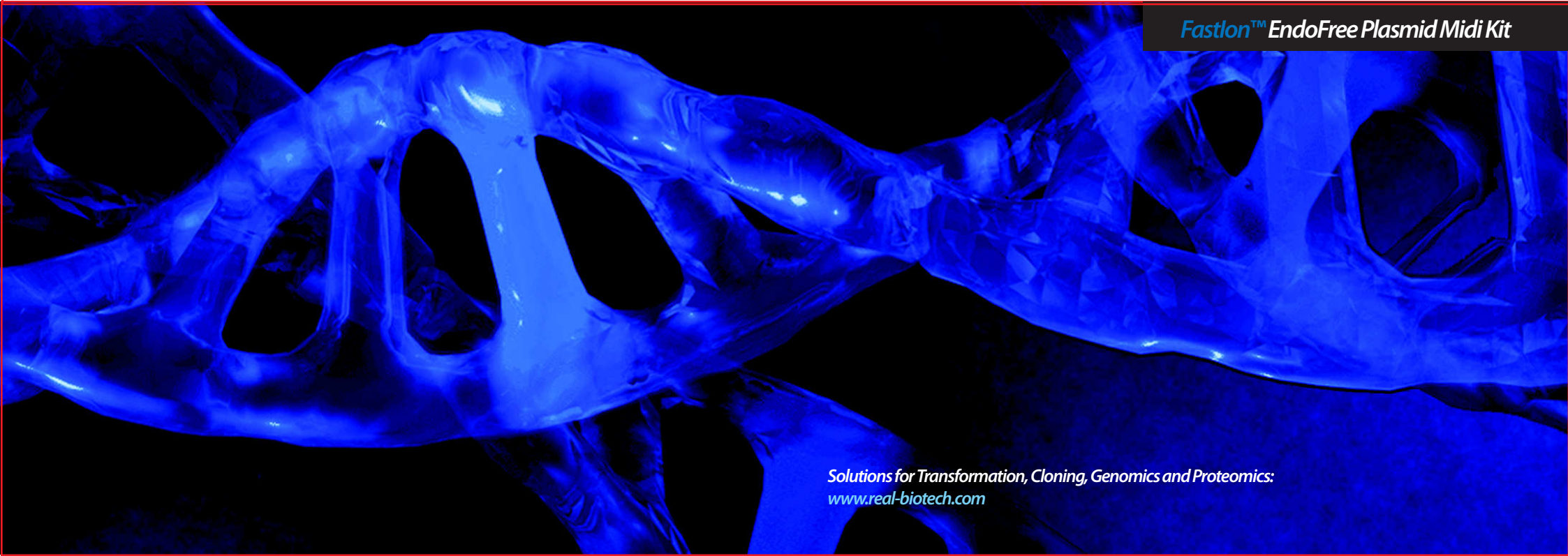
[2] If using water, ensure its pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification.

Troubleshooting

Problem	Possible Reasons/ Solution
Low yield	**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. Growth for more than 16 hours is not recommended. Use fresh bacterial cultures only. (≤16 hours incubated in a flask at 37˚C with 150-180 rpm shaking).
	Cell pellet was not resuspended completely Resuspend the cell pellet completely by vortex or pipette.
	Bacterial cells were not lysed completely Using bacterial culture (O.D.600 of 2-6) is recommended. Separate the culture into multiple tubes if necessary. Following PM3 Buffer addition, break up the precipitate by inverting or pipetting to ensure higher yield. Do not vortex.
	Incorrect DNA rehyrdation If using water for elution, ensure its pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification.
	No yield of plasmid DNA Using a single freshly isolated E. coli colony to inoculate into 50-100 ml of LB medium is recommended. Solid and liquid medium should contain antibiotics. Do not use overgrown bacterial cultures. Increase volume of low-copy number plasmid to 300 ml.
Eluted DNA does not perform well in downstream applications	**RNA contamination** Prior to using PM1 Buffer, ensure that RNase A was added. If RNase A added PM1 Buffer is out of date, add additional RNase A. After addition of RNase A and optional BlueMix Lysis Buffer, PM1 Buffer is stable for 6 months when stored at 2–8℃.
	Genomic DNA contamination Do not use overgrown bacterial culture. 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.

Notes

Fastlon™ EndoFree Plasmid Midi Kit



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