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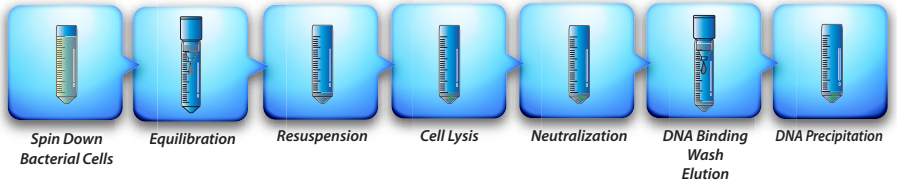
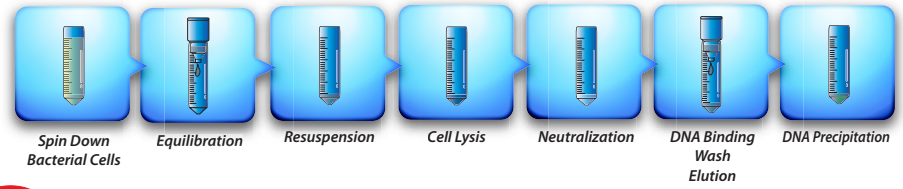
Real Genomics™

Store at room temperature 15°C~25°C

Workflow without using BlueMix Lysis Buffer



Workflow while using BlueMix Lysis Buffer



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FastIon™ EndoFree Plasmid Maxi Kit Protocol Book

Ultrapure Endotoxin-Free DNA from 200-1600 ml of Bacterial Cultures

Cat. No. **YPMF10, YPMF25**

Real Genomics™

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FastIon™ EndoFree Plasmid Maxi Kit

Fastlon™ EndoFree Plasmid Maxi Kit

Store at room temperature 15°C~25°C

Cat. No. **YPMF10**	Cat. No. **YPMF25**
10 maxi preps / kit	25 maxi preps / kit
PM1 Buffer: 110 ml *	PM1 Buffer: 275 ml *
PM2 Buffer: 110 ml **	PM2 Buffer: 275 ml **
PM3 Buffer: 110 ml	PM3 Buffer: 275 ml
BlueMix Lysis Buffer: 1.5 ml	BlueMix Lysis Buffer: 3 ml
PER Buffer: 40 ml	PER Buffer: 100 ml
PEQ Buffer: 130 ml	PEQ Buffer: 275 ml
PMC Buffer: 360 ml	PMC Buffer: 790 ml
PEL Buffer: 130 ml	PEL Buffer: 350 ml
RNase A (50mg/ml): 200 µl	RNase A (50mg/ml): 550 µl
PM Column: 10 pcs	PM Column: 25 pcs

Sample: 200-1600 ml of bacterial cultures (high-copy plasmid: 200-800 ml, low-copy plasmid: 500-1600 ml)
Yield: 1.0-1.2 mg of endotoxin-free, pure transfection grade plasmid DNA (1-20 kb) from 300 ml of cultured bacterial cells
Format: Anion-exchange resin column
Operation: 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 Maxi Kit is designed to purify pure transfection grade plasmid DNA (Endotoxin Free) from 200-1600 ml of cultured bacterial cells. More than 1mg of endotoxin-free plasmid DNA can be purified from 300 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 Maxi 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 more than 1mg of endotoxin-free plasmid DNA from 300 ml of cultured bacterial cells within 110 minutes.
BlueMix Lysis Buffer provides visual identification of optimum buffer mixing.
Phenol, chloroform or alcohol are not required.

Applications

Ultrapure, endotoxin-free, transfection grade plasmid DNA is ideal for plasmid DNA preparation, ligation, sequencing, in vitro transcription, PCR , restriction digestion, transfection, microinjection and gene gun.

Quality Control

The quality of Fastlon™ EndoFree Plasmid Maxi Kits are tested on a lot-to-lot basis by isolation of plasmid DNA from 300 ml overnight cultures of E.Coli DH5alpha containing the plasmid pBluescript (A_{600}>2 units/ml). Purified DNA is quantified with a spectrophotometer and the yield of plasmid DNA is more than 900 µg with A260/A280 ratio 1.8 to 2.0. The purified DNA is used in Eco R1 digestion and checked by electrophoresis.

Storage

Fastlon™ EndoFree Plasmid Maxi 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

⚠ DO NOT USE leftover PW Buffer. PW Buffer has been replaced with PMC Buffer. Please use only PMC Buffer with this kit.

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 dd$H_2$0.

Recommended culture volumes

1. High-copy plasmids: 3 g pellet wet weight; 800ml (OD600 = 2); 400ml (OD600 = 4); 250ml (OD600 = 6).
2. Low-copy plasmids: 6 g pellet wet weight; 1600ml (OD600 = 2); 800ml (OD600 = 4); 500ml (OD600 = 6).

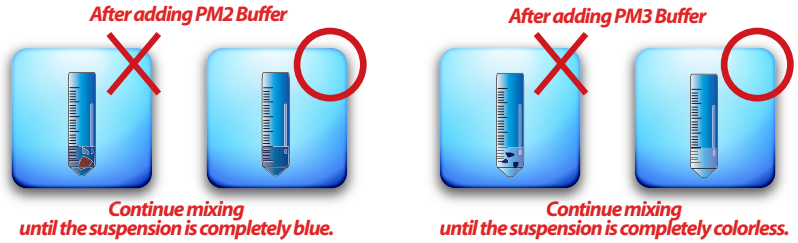
NOTE: For a higher yield, increase lysis buffer volumes by 1.5 times when using more than 3 g of cultured bacterial pellet. In this case, additional lysis buffer can be purchased from Real Biotech Corp.

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.

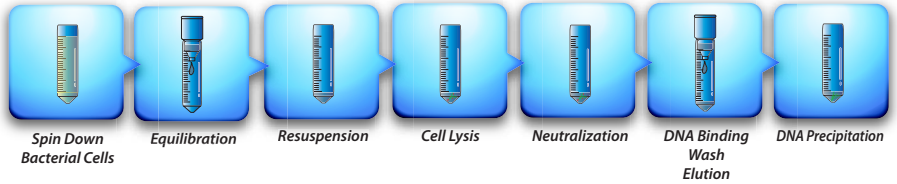


Protocol

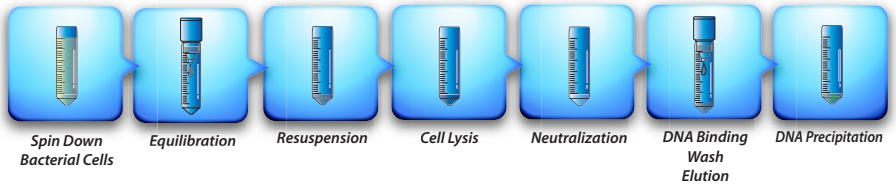
Please read the entire important notes before starting any of the protocol procedures.

This protocol is designed for rapid isolation of ultrapure endotoxin-free plasmid DNA from 200-1600 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 200-800 ml of high-copy plasmid or 500-1600 ml of low-copy plasmid cultured bacterial cells to a 50 ml centrifuge tube or a 250 ml centrifuge bottle 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 by using the same 50 ml centrifuge tube or 250 ml centrifuge bottle.

Equilibration

2. During centrifugation, place a PM Column in a new 50 ml centrifuge tube. Equilibrate the PM Column by adding 10 ml of PEQ Buffer. Allow the column to empty completely by gravity flow. Discard the flow-through and place the PM Column back in the 50 ml centrifuge tube then set it aside for DNA Binding Step.

Resuspension

3. Add 10 ml of PM1 Buffer (make sure RNase A was added) (Optional: Add 100 µl of BlueMix Lysis Buffer) to the 50 ml centrifuge tube or 250 ml centrifuge bottle 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:
- If the cell pellet is >3 g, add 15 ml of PM1 Buffer.
- If using a 250 ml centrifuge bottle, transfer the resuspended sample to a new 50 ml centrifuge tube.
- 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.

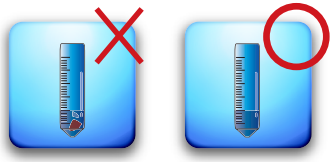
Cell Lysis

4. Add 10 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.

NOTE: If the cell pellet is >3 g, add 15 ml of PM2 Buffer.

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.



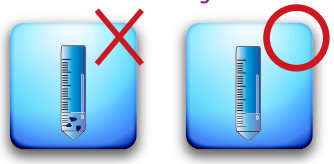
Neutralization

6. Add 10 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.

NOTE: If the cell pellet is >3 g, add 15 ml of PM3 Buffer.

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 3 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. Transfer the supernatant to the equilibrated PM Column. Allow the column to empty completely by gravity flow.
9. Discard the flow-through then place the PM Column back in the 50 ml centrifuge tube.

Wash

10. Wash the PM Column by adding 30 ml of PMC Buffer and allow the column to empty completely by gravity flow then discard the flow-through.

Elution

11. Place the PM Column in a clean 50 ml centrifuge tube then add 12 ml of PEL Buffer to elute the DNA by gravity flow. Discard the PM Column once it has emptied completely.

DNA Precipitation

12. Add 9 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.
13. 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 (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 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** Use flasks which are at least 3 times the volume of the culture medium to provide an oxygen saturated culture condition. 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 Use BlueMix Lysis Buffer (an color indicator) included in this kit to provide visual identification of optimum buffer mixing and prevent common handling errors. BlueMix Lysis Buffer is ideal for use by scientists who want to be assured of maximum product yield.
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 Maxi Kit