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PurElute™ IEX Plasmid Maxiprep

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Storage and Stability

All kit components can be stored at room temperature for up to 1 year. After dilution of RNase A and PurBlue solutions into Buffer S1, store the mixture at 4°C. RNase A/PurBlue/Buffer S1 Mix is stable at 4°C for six months. Store Buffer S3 and Buffer S4 at 4°C after opening.

Kit Components

PurElute™ IEX Plasmid Maxiprep Kit	**(2) 91006**	**(10) 91008**
IEX Maxi Column	2	10
Lysate Filtration Units	2	10
Buffer S1 (Resuspension)	25 ml	120 ml
RNase A (100mg/ml)	30 µl	150 µl
PurBlue™ Solution	25 µl	120 µl
Buffer S2 (Lysis)	25 ml	120 ml
Buffer S3 (Neutralization)	25 ml	120 ml
Buffer S4 (Extraction)	7.5 ml	30 ml
Buffer C5 (Wash)	40 ml	2 × 90 ml
Buffer C6 (Elution)	15 ml	60 ml
Endotoxin-Free Water	4.5 ml	17 ml
TE Buffer	5 ml	20 ml

Quality Control

Final endotoxin level of purified DNA is tested by *Limulus* Amebocyte Lysate (LAL) assay with pyrogent single test (Lonza, Walkersville, MD).

Introduction

The PurElute™ IEX Plasmid Maxiprep was developed for the purification of superior quality endotoxin-free plasmid DNA. IEX columns are made of a unique anion-exchange membrane which has an exceedingly high binding capacity. Purified plasmid DNA is free of impurities that may interfere with demanding downstream applications such as enzymatic modifications, transfection into sensitive eukaryotic cells, gene therapy research, RNA silencing and in vitro transcription/translation.

Benefits

- **Superior Quality DNA** – Suitable for mammalian cell transfections and other sensitive downstream applications
- **High Purity DNA** – Endotoxin-free
- **High Yield** – Binding capacity of ≥1 mg of plasmid DNA
- **User-Friendly** – Simple spin column format
- **PurBlue™** – Visualize complete lysis for maximum DNA yield

Features of the PurElute™ IEX Plasmid Maxiprep

Column binding capacity	1 mg plasmid DNA
Culture volume	100-250 ml
Yield (depends on plasmid size and copy number, bacterial host, and culture volume)	Up to 1 mg
A_{260}/A_{280}	1.85-1.99
Endotoxin level	< 0.01 EU/ µg DNA
RNA contamination (agarose gel analysis)	Not visible

Before Starting

Equipment and Additional Materials Needed

- Refrigerated bench-top centrifuge with swinging bucket
- Refrigerated centrifuge with a centrifugation force limit > 15,000 × g
- Nonpyrogenic disposable 50 ml conical centrifuge tubes
- Nonpyrogenic disposable 14 ml round bottom centrifuge tubes
- Nonpyrogenic plasticware: pipettes, pipette tips, microcentrifuge tubes, etc.
- Isopropanol
- Ethanol (96-100%)

Bacterial Cultures

Inoculate 100 - 200 ml of Luria-Bertani (LB) containing the appropriate antibiotic with a single colony or 5 µl of glycerol stock. Incubate at 37°C while shaking at 300 rpm for approximately 16 hours.

- To ensure good aeration of the culture and to achieve optimal bacterial growth it is recommended to use a flask with a volume of 4 to 5 times that of the culture volume.
- Optimal OD_{600} of the overnight LB culture is approx. 3.0.
- Starting volume can be adjusted based on the amount of plasmid DNA needed, the size of the plasmid, and copy number of the plasmid.

Culture Volume / Suggested Volume for Buffers

LB culture volume[§]	**100 ml**	**150 ml**	**200 ml**
Buffer S1	7.5 ml	10 ml	12.5 ml
Buffer S2	7.5 ml	10 ml	12.5 ml
Buffer S3	7.5 ml	10 ml	12.5 ml
Approx. Vol. of Cleared Lysate	18 ml	25 ml	30 ml

[§] For culture volume ≥ 250 ml, refer to "Protocol for large volume bacterial lysis" on page 9.

Prepare RNase A/PurBlue/Buffer S1 Mix

Centrifuge RNase A and PurBlue™ vials briefly before opening. Transfer the entire content of both RNase A and PurBlue™ to the bottle of Buffer S1 and mix well. Label bottle 'RNase/PurBlue™ added'. Store any unused RNase A/PurBlue™/Buffer S1 mix at 4°C for up to six months.

Prepare 70% Ethanol

Prepare 70% Ethanol by adding 7 volumes of ethanol into 3 volumes of endotoxin-free water in the bottle.
Label bottle 'ethanol added'.

Endotoxin-Free Water	Ethanol
4.5 ml	10.5 ml
17 ml	40 ml

General Information

1. We recommend the use of endotoxin-free or non-pyrogenic pipette tips, centrifuge tubes, and reagents after lysate loading step to avoid introducing endotoxins into purified plasmid DNA.

2. Do not steam autoclave pipet tips, centrifuge tubes, or reagents as it causes contamination and does not destroy endotoxins.

3. Store Buffer S3 and Buffer S4 at 4°C after opening.

4. Pre-chill Buffer S3 and Buffer S4 on ice before use.

5. 5 ml elution in step 17 can be aliquoted into several micro-centrifuge tubes.

Recommended Protocol

1. **Inoculate 100 - 200 ml of LB containing the appropriate antibiotic with a single colony or 5 µl of glycerol stock. Incubate at 37°C while shaking at 300 rpm for approximately 16 hours.**

2. **Pellet cells by centrifugation at 5,000 × g for 15 minutes at 4°C.**

3. **Place Buffer S3 and Buffer S4 on ice.**

4. **Label two 50 ml conical tubes 'cell lysis' and 'cleared lysate'. Place the 'cleared lysate' tube on ice.**

5. **Remove pelleted culture from the centrifuge and discard the supernatant by decanting.**
   - Leave the tube inverted on a clean absorbent pad for a few minutes prior to continuing to ensure all excess supernatant is removed.

6. **Resuspend cells thoroughly in an appropriate volume of RNase A/PurBlue™/Buffer S1 Mix (based on the volume of culture being processed. See table on page 4). Mix by vortexing or pipetting up and down.**
   - Ensure RNase A/PurBlue™/Buffer S1 is mixed well by inverting briefly before use.

7. **Transfer the resuspension to the clean 50 ml tube labeled 'cell lysis'.**

8. **Start a timer for 5 minutes. Add an appropriate volume of Buffer S2 to lyse the cells (based on the volume of culture being processed. See table on page 4). Mix gently by inverting until homogeneous. Incubate at room temperature for the remainder of the 5 minutes on the timer.**
   - Start rapidly inverting by hand, then reduce the speed as the mixture becomes more viscous to avoid foaming.
   - Vigorous mixing may shear chromosomal DNA.
   - The color of the mixture will change to a homogeneous blue color when lysis is complete.

9. **Add an appropriate volume of ice-cold Buffer S3 (based on the volume of culture being processed. See table on page 4). Mix gently but thoroughly by slowly inverting and rotating the tube to neutralize the reaction.**
    - Avoid vigorous mixing; gentle mixing will help keep the precipitate intact, which facilitates filtration.
    - If precipitate breaks into many small pieces it may clog the lysate filtration unit.
    - During neutralization the blue viscous aggregate turns into a light and fluffy precipitate.
    - Lysate and precipitate change color from blue to clear and white respectively.

10. **Incubate on ice for 20 minutes.**
    - Lowering the temperature enhances precipitation and allows the precipitates to aggregate together.
    - After neutralization the solution should be clear and most of the precipitates form flocculants which will be floating on the top.
    - If most of the precipitates failed to aggregate and are not floating on top, transfer the crude lysate into a 50 ml centrifuge tube and spin at 20,000 × g for 10 minutes at 4°C.

11. **Pull the plunger out of the filtration unit. Keeping the barrel at a slight angle slowly pour the entire mixture into the lysate filtration unit without disturbing the aggregates.**

12. **Insert the plunger into the filtration unit and filter the lysate into the clean tube on ice labeled 'cleared lysate'.**
    - Aim the tip of the filter at the clean tube before inserting the plunger.

13. **Add approx. 0.1 volume of ice-cold Buffer S4 to the cleared lysate and mix by inverting 10 times. Place the tube on ice.**

14. **Transfer the treated lysate into the IEX Maxi Column with collection tube and centrifuge at 650 × g (2,000 rpm) for 5 minutes at 4°C.**

15. **Discard flow through and repeat step 14 until all of the lysate has been processed.**

16. **Add 16 ml of Buffer C5 to the IEX Maxi Column with collection tube. Centrifuge at 650 × g (2,000 rpm) for 5 minutes at 4°C. Discard flow through.**

17. **Transfer the washed column into a clean 50 ml tube and add 5 ml of Buffer C6 to elute. Centrifuge at 650 × g (2,000 rpm) for 5 minutes at 4°C.**

18. **Transfer the 5 ml eluate to a clean 14 ml centrifuge tube.**
    - A nonpyrogenic 14 ml tube that can be centrifuged at 15,000 × g is recommended.
    - Alternatively, aliquot the eluate into several 1.5 ml microcentrifuge tubes (~850 µl/tube)

19. **Precipitate DNA by adding 3.5 ml of isopropanol to the 5 ml eluate and mix. Centrifuge at 15,000 × g for 15 min at 4°C.**
    - If microcentrifuge tubes were used, add 0.7 volumes of isopropanol per tube, mix, and centrifuge at 15,000 × g for 15 minutes at 4°C.

20. **Discard supernatant carefully by decanting or pipetting.**
    - DNA pellet is usually visible.
    - After decanting, a clean paper towel may be used to absorb the excess supernatant from the mouth of the tube.

21. **Add 5 ml of 70% ethanol solution to rinse the pellet. Centrifuge at 15,000 × g for 10 minutes at 4°C.**
    - Do not vortex.
    - If microcentrifuge tubes were used, add 500 µl of 70% ethanol solution to each and centrifuge at 15,000 × g for 10 minutes at 4°C.

22. **Discard supernatant carefully by decanting or pipetting.**
    - After decanting, a clean paper towel may be used to absorb the excess supernatant from the mouth of the tube.

23. **Air-dry DNA pellet for 10 minutes to ensure all ethanol has evaporated.**

24. **Add 250 µl - 500 µl of TE Buffer (provided) or endotoxin-free water to dissolve the DNA.**
    - If microcentrifuge tubes were used, divide a total of 250 to 500 µl of TE Buffer or endotoxin-free water among all tubes to dissolve the DNA.

Protocol for Large Volume Bacterial Lysis

The PurElute™ Plasmid Maxiprep has an exceedingly high DNA binding capacity of 1 mg plasmid DNA. A higher yield can be obtained by loading more cleared lysate from a large volume of LB culture (≥ 250 ml).

For culture volume ≥ 250 ml in a flask, we recommend first growing a 2 ml starter culture for approx. 8 hours, then inoculating 1:500 into growth medium for overnight culture.

1. **Pellet cells by centrifugation at 5,000 × g for 15 minutes at 4°C.**
    - Use one 250 ml centrifuge tube for every 200 ml of LB culture.

2. **Place Buffer S3 and Buffer S4 on ice.**

3. **Remove pelleted culture from the centrifuge and discard the supernatant by decanting.**
    - Leave the tube inverted on a clean absorbent pad for a few minutes prior to continuing to ensure all excess supernatant is removed.

4. **Resuspend cells thoroughly in 0.06 volume of RNase A/PurBlue/Buffer S1 Mix. Mix by pipetting up and down.**
    - If more than one centrifugation tube was used, combine the resuspension into one tube and proceed.

5. **Start a timer for 5 minutes, add the same volume of Buffer S2 as RNase A/PurBlue™/Buffer S1 and mix well by swirling until homogeneous to lyse the cells. Incubate at room temperature for the remainder of the 5 minutes on the timer.**
    - The color of the mixture will change to a homogeneous blue color when lysis is complete.

6. **Add the same volume of ice-cold Buffer S3 and mix by swirling to neutralize the reaction.**
   - Lysate and precipitate change color from blue to clear and white respectively.

7. **Incubate on ice for 20 minutes.**

8. **Centrifuge at 20,000 × g for 10 minutes.**

9. **Pull the plunger out of the filtration unit. Keeping the barrel at a slight angle slowly pour the supernatant into the lysate filtration unit.**

10. **Insert the plunger into the filtration unit and filter the lysate into clean 50 ml conical tube(s) on ice labeled 'cleared lysate'.**
    - Aim the tip of the filter at the clean tube before inserting the plunger.
    - Filter excess supernatant by re-using the same lysate filtration unit. Unscrew the filter from the syringe and draw out the plunger. Reconnect the filter to the syringe and add excess supernatant to filter.

11. **Proceed to step 13 of the recommended protocol.**

Troubleshooting Guide

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
Poor DNA yield	OD_{600} is too high or too low	Make sure the OD_{600} of the culture is approx. 3.0. Use fresh materials and bacterial colonies to ensure sufficient growth. Ensure the concentration of antibiotics and incubation time is adequate. If culture volume is larger than 250 ml, grow a 2 ml starter culture for 8 hours then inoculate 1:500 into large volume of media and grow overnight for 16 hours.
	Failure to pellet all cells	Increase centrifugation force or time.
	Failure to fully resuspend cells	Decrease centrifugation force or time to keep cell pellet from getting too tight. Increase pipetting time until pellet is completely dispersed.

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
Poor DNA yield *cont.*	Insufficient cell lysis	Resuspend cells completely. If there is SDS precipitation in Buffer S2, warm at 37°C until dissolved then cool down to room temperature. After the addition of Buffer S2, mix by gently inversion until the mixture becomes clear and homogenous, i.e. no clouds of cell.
	Failure to precipitate DNA or lost DNA pellet	Make sure the appropriate amount of isopropanol is added to precipitate the DNA. Make sure the final concentration of ethanol is correct. Check centrifugation speed and time to ensure they are correct. Use a disposable 14 ml BD round bottom tube (Fisher 14-959-10B) for DNA precipitation. DNA pellet should be visible.
	Failure to dissolve DNA pellet	To avoid over-drying of DNA pellet, decrease drying time and air-dry at room temperature not under a vacuum.

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
Poor DNA yield *cont.*	Failure to dissolve DNA pellet *cont.*	Increase the incubation time for dissolving DNA. If endotoxin-free water is used for dissolving DNA, use fresh water with pH close to 7.
RNA contamination	Insufficient RNA digestion	Make sure RNase A has been added into Buffer S1. Store RNase A/PurBlue™/ Buffer S1 mixture at 4°C for no longer than 6 months.
Genomic DNA contamination	Vigorous mixing or too long of an incubation time in cell lysis step	Mix gently but thoroughly keeping the whole lysis process no longer than 5 minutes.
Endotoxin contamination higher than expected	Incomplete neutralization or incubation	After the addition of Buffer S3, mix gently by inverting and rotating the tube slowly many times until lysate and precipitates change color to clear and white respectively. Make sure the cleared lysate was placed on ice during the steps prior to loading on IEX Maxi Column.

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
Endotoxin contamination higher than expected *cont.*	Use of materials contaminated by endotoxins	Make sure all the plasticware and solutions are not contaminated by using nonpyrogenic grade plasticware. Do not sterilize items by steam autoclaving.
Difficulty to filter or low volume recovery	An excess of small debris in lysate blocking the flow.	After the addition of Buffer S3, mix gently by inverting the tube slowly many times until lysate and precipitates change color to clear and white respectively. Centrifuge at 20,000 × g for 10 min then transfer the supernatant into the filtration unit.

Note: For new users of the kits, we strongly suggest saving an aliquot of each separation fraction for possible troubleshooting analysis. Save an aliquot of 50 µl of the cleared lysate and DNA eluate and 0.5 ml of each flow through fraction from IEX column, including flow through during sample loading, column washing, and the supernatants. Recover DNA from the fractions by isopropanol precipitation. Rinse the pellets with 70% ethanol and centrifuge. After evaporation of the ethanol, dissolve with 10 µl of TE buffer and analyze with agarose gel electrophoresis.

Frequently Asked Questions

About your PurElute™ IEX Maxiprep Kit

What is the PurElute™ IEX Maxiprep Kit?

The PurElute™ IEX Plasmid Maxiprep Kit is a high performance, high yield plasmid purification kits based on a novel ion exchange membrane (IEX) technology, which is easier to process and more efficient than conventional gravity flow-based methods. Purified plasmid DNA is free of impurities that may interfere with demanding downstream applications, such as enzymatic modifications, transfection into sensitive eukaryotic cells, gene therapy research, gene silencing, and in vitro transcription/translation.

How does the kit work?

Plasmid DNA is extracted from bacterial cells through alkaline lysis. The crude lysate is cleared by a sequential filtration device that not only removes cell debris but micellar aggregates as well. The cleared lysate is mixed with an extraction solution and loaded onto a column containing an IEX membrane. Binding, washing, and elution conditions are optimized to achieve efficient isolation of plasmid DNA from impurities. IEX columns have an exceedingly high dynamic binding capacity, excellent selectivity and a near 100% recovery for plasmids.

What is the binding capacity of the IEX columns?

The estimated dynamic binding capacity for pUC19 is 2.93 mg pUC19 per cm^3 of the IEX membrane. This is about ten times greater than the typical capacity for conventional gravity flow columns. The total tested binding capacity of the PurElute™ IEX Maxiprep column for pUC19 is approx. 1.7 mg.

How do I store the PurElute™ IEX Plasmid Maxiprep Kit?

The PurElute™ IEX Plasmid Maxiprep Kit is shipped at an ambient temperature, and can be stored at room temperature until use. All the buffers and RNase A stock solution are stable for 1 year at room temperature.

RNase A/PurBlue is stable for 6 months at 4°C after dilution into Buffer S1.

Store Buffer S3 and Buffer S4 at 4°C after opening.

Prepare all solutions before use and store appropriately.

What DNA recovery efficiency and total yield should I expect from the PurElute™ IEX Plasmid Maxiprep Kit?

The DNA recovery is close to 100%. The total DNA yield obtained from the PurElute™ IEX Plasmid Maxiprep Kit is dependent on plasmid size and copy number, bacterial host and the amount of culture used in the preparation.
The expected yield from a 200 ml LB culture using the PurElute™ IEX Plasmid Maxiprep Kit is 200-500 µg for high copy plasmids and 50-200 µg for low copy plasmids. To increase the yield, the volume of the culture can be increased.

What DNA purity and quality should I expect from the PurElute™ IEX Plasmid Maxiprep Kit?

Endotoxin level in a DNA sample prepared with the PurElute™ IEX Plasmid Maxiprep Kit is expected to be <0.01 EU/µg because of the use of the distinct sequential filtration device and the unique highly selective IEX matrix. The endotoxin level is >10 times below 0.1 EU/µg, which is considered "endotoxin-free".

How can I quantify plasmid DNA purified with the PurElute™ IEX Plasmid Maxiprep Kit?

PurElute™ IEX Plasmid Maxiprep Kit purified plasmid DNA can be accurately quantified by using UV spectrophotometry, agarose gel analysis or Invitrogen's Quant-iT™ PicoGreen® dsDNA Assay Kit. There is no significant difference among DNA concentrations determined by these methods due to the superior purity of DNA prepared with the PurElute™ IEX Plasmid Maxiprep Kit.

Bacterial Strains and Vector Types

Which *E. coli* strains work with the PurElute™ IEX Maxiprep Kit?

Most conventional *E. coli* strains used in the lab will work with PurElute™ IEX Maxiprep Kits.

Which plasmid vectors are suitable for the PurElute™ IEX Maxiprep Kit?

PurElute™ IEX Plasmid Maxiprep Kits can isolate most types of plasmids, including high-copy and low-copy plasmids, cosmids, fosmids, BACs, etc.

Bacterial Growth and Cell Lysis

What is the recommended bacterial growth medium?

Luria-Bertani (LB) is recommended for the overnight bacterial culture. Optimal OD_{600} of the overnight LB culture is about 3.0. Rich media such as 2×YT or Terrific Broth (TB) are not recommended but can be used. Adjust culture volume used in purification based on cell density in the culture. For example, if OD_{600} of an overnight culture is 6.0, use half of the volume of the culture indicated in user's protocol.

Luria-Bertani (1 Liter)
10 g Tryptone
5 g Yeast Extract
10 g NaCl

Add dH_2O to one liter and autoclave.

What should a normal lysis process look like?

After lysis and neutralization, the lysate should be clear and the precipitate white, fluffy and intact.
A cloudy or turbid lysate after lysis indicates an incomplete lysis. Complete mixing by gentle inversion and slow rotation of the tube during neutralization was found to be critical for keeping the precipitate intact. An excess of small debris will cause difficulty in filtration. Incubation of the neutralized lysate in ice resulted in

better aggregation of precipitates and removal of contaminants as compared to incubation at room temperature.

What is the PurBlue™ Solution?

PurBlue Solution contains a pH indicator, which allows you to visualize the alkaline lysis process. PurBlue ensures sufficient mixing during the lysis and neutralization steps to guarantee the highest yield.

After the addition of Buffer S2 for cell resuspension, the mixture changes color to a homogeneous blue. After neutralization of the lysed cells with Buffer S3, the color of the lysate and precipitates changes to clear and white respectively.

DNA Preparation – IEX Chromatography

What are the performance characteristics for IEX spin columns?

The porosity was optimized to provide greater interior surface area for plasmid binding and high flow rate for easy sample processing. The spin column is stored dry and ready to use without a pre-wetting or equilibration step. Due to the exceedingly high dynamic binding capacity and the high flow rate, buffer volume and operation time for each step are considerably less than most conventional ion-exchange resin-based methods.

Do I need to equilibrate IEX column before use?

No. IEX spin columns are stored dry and ready to use without a pre-wetting or equilibration step. There is no equilibration buffer in the kits.

Can I use vacuum or gravity to drive IEX chromatography?

No. IEX columns are designed in a spin column format. Due to the unique microporous structure of the IEX membrane, the back pressure for the spin columns is very low. Centrifugation can be done almost instantly with minimal volume of retention. In contrast, using a vacuum may cause over-drying of the column. Conventional gravity columns have much lower flow rates and larger retention of DNA than IEX columns.

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