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PurElute™ IEX Plasmid Miniprep

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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

PurElite™ IEX Plasmid Miniprep Kit	(5) 91002	(25) 91004
IEX Mini Column	5	25
Buffer S1 (Resuspension)	3 ml	10 ml
RNase A (20 mg/ml)	30 µl	150 µl
PurBlue™ Solution	10 µl*	10 µl
Buffer S2 (Lysis)	3 ml	10 ml
Buffer S3 (Neutralization)	3 ml	10 ml
Buffer S4 (Extraction)	2 ml	3 ml
Buffer C5 (Wash)	3 ml	15 ml
Buffer C6 (Elution)	3 ml	15 ml
Endotoxin-Free Water	0.9 ml	4.5 ml
Tris Buffer (10mM Tris-HCl, pH 8.0)	2 ml	5 ml

*10 µl of PurBlue™ is provided in the PurElite™ IEX Plasmid Miniprep Kit (5), transfer ONLY 3 µl of PurBlue into 3 ml of Buffer S1, i.e. a 1:1000 dilution.

Quality Control

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

Introduction

The PurElute™ IEX Plasmid Miniprep was developed for the purification of superior quality transfection grade 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, gene silencing and in vitro transcription/translation.

Benefits

- **Superior Quality DNA** – Suitable for mammalian transfection and other sensitive downstream applications
- **High Purity DNA** – Transfection grade
- **High Yield** – Binding capacity of $\geq 50 \mu\text{g}$ of plasmid DNA
- **User-Friendly** – Simple spin column format
- **PurBlue™** - Visualize complete lysis for maximum DNA yield

Features of the PurElute™ IEX Plasmid Miniprep

Column binding capacity	$\geq 50 \mu\text{g}$ plasmid DNA
Culture volume	1-5 ml
Yield (depends on plasmid size and copy number, bacterial host, and culture volume)	Up to $50 \mu\text{g}$
A_{260}/A_{280}	1.81-1.99
Endotoxin level	0.1-1 EU/ μg DNA
RNA contamination (agarose gel analysis)	Not visible

Before Starting

Equipment and Additional Materials Needed

- Microcentrifuge with a centrifugation force limit > 15,000 g; Refrigerated microcentrifuge recommended
- Isopropanol
- Ethanol (96-100%)
- Recommended for highest purity DNA: Nonpyrogenic plasticware including; pipettes, pipette tips, microcentrifuge tubes, etc.

Prepare RNase A/PurBlue™/Buffer S1 Mix

- Centrifuge RNase and PurBlue vials briefly before opening.
- For the PurElute™ IEX Plasmid Miniprep Kit (5), transfer only 3 µl of PurBlue into the bottle of 3 ml of Buffer S1.
- For the PurElute™ IEX Plasmid Miniprep Kit (25), transfer the entire content of PurBlue™ into the bottle of Buffer S1.
- Label bottle 'RNase/PurBlue™ added'. Store 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
0.9 ml	2.1 ml
4.5 ml	10.5 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.
4. Pre-chill Buffer S3 and Buffer S4 on ice before use.

Recommended Protocol

- 1. Inoculate 1 - 5 ml of LB containing the appropriate antibiotic with a single colony or 5 μ l of glycerol stock. Incubate at 37°C while shaking at 300 rpm for approximately 16 hours.**
- 2. Pellet cells by centrifugation at 5,000 \times g for 5 minutes at room temperature.**
- 3. Place Buffer S3 and Buffer S4 on ice.**
- 4. Remove pelleted culture from the centrifuge and discard the supernatant by decanting.**
 - Tap lightly on a clean absorbent pad to ensure all excess supernatant is removed.
- 5. Add 0.3 ml of RNase A/PurBlue™/Buffer S1. Resuspend cells thoroughly by vortexing.**
 - Mix RNase A/PurBlue™/Buffer S1 well by inverting briefly before use.
- 6. Start a timer for 5 minutes. Add 0.3 ml of Buffer S2 to lyse the cells. Mix gently by inverting 10 times or until homogeneous. Incubate at room temperature for the remainder of the 5 minutes on the timer.**
 - The color of the mixture will change to clear blue.
 - Avoid vigorous mixing which may shear chromosomal DNA.
- 7. Add 0.3 ml of ice-cold Buffer S3 and mix well by inverting to neutralize the reaction. Incubate on ice for 5 minutes.**
 - Lysate and precipitate will change color from blue to clear and white respectively.
 - Lowering the temperature enhances precipitation of impurities.
- 8. Centrifuge at 15,000 \times g for 10 minutes at 4°C and transfer supernatant to a clean tube.**
 - It is recommended to perform centrifugation at 4°C, however room temperature centrifugation is acceptable.
- 9. Add 90 μ l of ice-cold Buffer S4 to the cleared lysate and mix by inverting 10 times. Place the tube on ice.**

10. **Transfer 500 μ l treated lysate into the IEX Mini column with collection tube and centrifuge at 500 \times g for 3 minutes at room temperature. Discard the flow-through.**
11. **Repeat step 10 until all of the lysate has been processed.**
12. **Add 0.5 ml of Wash Buffer C5 to the IEX Mini column with collection tube and centrifuge at 500 \times g for 3 minutes at room temperature. Discard the flow-through.**
13. **Transfer the column into a clean tube and add 0.5 ml of Buffer C6 to elute. Centrifuge at 500 \times g for 3 minutes at room temperature.**
14. **Precipitate DNA by adding 0.35 ml of isopropanol to the eluate and mixing. Centrifuge at 15,000 \times g for 15 min at 4°C.**
 - It is recommended to perform centrifugation at 4°C, however room temperature centrifugation is acceptable.
15. **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.
16. **Add 0.5 ml of 70% ethanol to rinse the DNA pellet. Centrifuge at 15,000 \times g for 10 minutes at 4°C.**
 - It is recommended to perform centrifugation at 4°C, however room temperature centrifugation is acceptable.
 - Do not vortex before centrifugation.
17. **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.
18. **Air-dry DNA pellet for 10 minutes to ensure all ethanol has evaporated.**
19. **Add 50 μ l of endotoxin-free Tris Buffer (provided) or endotoxin-free water to dissolve the DNA.**

Troubleshooting Guide

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
Poor DNA yield	OD ₆₀₀ is too high or too low	<p>Make sure the OD₆₀₀ of the culture is about 3.0.</p> <p>Use fresh materials and bacterial colonies to ensure sufficient growth.</p> <p>Ensure the concentration of antibiotics and incubation times are adequate.</p>
	Failure to pellet all cells	Increase centrifugation force or time.
	Failure to fully resuspend cells	<p>Decrease centrifugation force or time to keep cell pellet from getting too tight.</p> <p>Increase vortexing time until pellet is completely dispersed.</p>
	Insufficient cell lysis	<p>Resuspend cells completely.</p> <p>If there is SDS precipitation in Buffer S2, warm at 37°C until dissolved then cool down to room temperature.</p> <p>After the addition of Buffer S2, mix by gentle inversion until the mixture becomes blue and homogenous, i.e. no clouds of cell.</p>

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
<p>Poor DNA yield cont.</p>	<p>Failure to precipitate DNA or lost DNA pellet</p>	<p>Make sure the appropriate amount of isopropanol was added.</p> <p>Make sure the final concentration of ethanol is correct.</p> <p>Check centrifugation speed and time to ensure they are correct.</p> <p>Mark the orientation of the tube during centrifugation so that you can avoid touching the pellet site when pipetting out supernatant.</p>
	<p>Failure to dissolve DNA pellet</p>	<p>To avoid over-drying of DNA pellet, decrease drying time and air-dry at room temperature instead of under a vacuum.</p> <p>Increase the incubation time for dissolving DNA.</p> <p>If Endotoxin-Free water is used for dissolving DNA, use fresh water with pH close to 7.0.</p>
<p>RNA contamination</p>	<p>Insufficient RNA digestion</p>	<p>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.</p>

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
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	Transfer of cell debris into IEX column	Repeat step 8, centrifugation of the neutralized lysate to remove all debris.
	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.

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 μ l 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 μ l of TE buffer and analyze with agarose gel electrophoresis.

Frequently Asked Questions

About your PurElute™ IEX Plasmid Miniprep Kit

What is the PurElute™ IEX Plasmid Miniprep Kit?

The PurElute™ IEX Plasmid Miniprep Kit is a high performance, high yield plasmid purification kit based on a novel ion exchange membrane (IEX) technology, which is easier to process and more efficient than conventional gravity column-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 centrifugation, mixed with an extraction solution and loaded onto a spin 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 cm³ of the IEX membrane. This is about 10 times greater than the typical capacity for conventional ion exchange beads. The total tested binding capacity of the PurElute™ IEX Miniprep column for pUC19 is approximately 167 µg.

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

The PurElute™ IEX Plasmid Miniprep Kit is shipped at 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 is stable for 6 months at 4°C after dilution into Buffer S1. Prepare all solutions before use, and store appropriately.

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

The DNA percent recovery is close to 100%. The total DNA yield obtained from the PurElute™ IEX Plasmid Miniprep Kit is dependent on plasmid size and copy number, bacterial host and growth medium used in the preparation.

The expected yield using the PurElute™ IEX Plasmid Miniprep Kit is 10-50 µg for high copy plasmids and 5-10 µg for low copy plasmids.

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

The endotoxin level in a DNA preparation with the PurElute™ IEX Plasmid Miniprep Kit is in the range of 0.1 - 1.0 EU/µg DNA. Purified DNA is of transfection-grade quality.

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

PurElute™ IEX Plasmid Miniprep 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 Miniprep Kit.

Bacterial Strains and Vector Types

Which *E. coli* strains work with PurElute™ IEX Plasmid Miniprep Kits?

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

Which plasmid vectors are suitable for PurElute™ IEX Plasmid Miniprep Kits?

PurElute™ IEX Plasmid MiniPrep 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 OD₆₀₀ 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 OD₆₀₀ 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 dH₂O to one liter and autoclave.

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 of the IEX spin column?

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. The spin columns are configured to fit in standard microcentrifuge tubes. 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. Gravity columns have much lower flow rates and larger retention of DNA than IEX columns.

Will a second DNA elution from the IEX columns yield more DNA?

No. IEX columns have a very sharp elution peak. A single elution is enough to recover nearly 100% of the DNA.

DNA Preparation – DNA Precipitation and Redissolving

What is the recommended buffer for dissolving DNA pellet?

Endotoxin-free water, 10mM Tris-HCl, pH 8.0, or TE can be used to dissolve DNA pellet. EDTA in TE buffer may inhibit downstream applications in enzymatic reactions. Endotoxin free 10mM Tris-HCl, pH 8.0 is provided in the PurElute™ IEX Miniprep Kit. Endotoxin free water included in the kits is for preparing 70% ethanol.

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