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PureLink™ Expi Endotoxin-Free Maxi Plasmid Purification Kit

USER GUIDE

For rapid, large scale purification of endotoxin-free plasmid DNA

Catalog Numbers A33073, A31217, A31231

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Revision A.0

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ThermoFisher SCIENTIFIC

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Revision	Date	Description
A.0	30 March 2017	New manual

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Product description

The PureLink™ Expi Endotoxin-Free Maxi Plasmid Purification Kit enables isolation of large (milligram) quantities of highly pure, endotoxin-free (<0.1 EU/µg) advanced transfection-grade plasmid DNA for use in your most sensitive downstream applications.

The DNA Binding Column utilizes a unique, proprietary, enhanced anion exchange membrane that enables rapid, large scale purification of endotoxin-free plasmid DNA, in as little as 90 minutes (including the precipitation step).

Endotoxin is a component of the plasma membrane of gram negative bacteria (e.g., *E. coli*), and a common contaminant in plasmid preparations. Endotoxin levels are reported as endotoxin units per microgram of plasmid DNA (EU/µg DNA).

Samples with <0.1 EU/µg are considered to be endotoxin free, but high endotoxin levels (>10 EU/µg) can severely reduce cell viability and gene expression, and lead to activation of innate cellular immune responses.

In order to attain endotoxin free plasmid purifications, specialized kits are required. Unlike silica-membrane resins, the anion exchange membrane used in PureLink™ Expi plasmid purification kits produce plasmid DNA with inherently low endotoxin levels of 0.1–1.0 EU/µg. Endotoxin-free components are incorporated into the kit, and a proprietary Endotoxin Removal Buffer further enhances endotoxin depletion, resulting in plasmid DNA that is consistently endotoxin-free.

Sensitive applications such as transfection of primary, immune, or neuronal cells, *in vivo* experiments, or gene therapy and plasmid DNA vaccine research, require endotoxin free plasmid DNA, which can be conveniently produced using the PureLink™ Expi Endotoxin-Free Maxi Plasmid Purification Kit.

Advantages of the PureLink™ Expi Endotoxin-Free Maxi Plasmid Purification Kit

- **High Yield**
  Isolate up to 1.5 mg of high quality endotoxin-free plasmid DNA from 100–200 mL of bacterial culture.
- **Purity**
  Produce endotoxin free (<0.1 EU/µg) plasmid preparations ideal for sensitive applications such as transfection in primary, immune, or neuronal cells, *in vivo* experiments, or gene therapy and plasmid DNA vaccine research.
- **Simple and fast protocol**
  Unique centrifuge and vacuum assisted protocols with advanced membrane technology enable the fastest anion exchange purifaction, and allow a typical maxi plasmid isolation to be performed in as little as 90 minutes.
  Lysis Indicator turns blue during cell lysis and helps to ensure complete lysis and subsequent neutralization.

System overview

To isolate plasmid DNA using the kit, grow transformed *E. coli* cells overnight and harvest by centrifugation. Resuspend the cells in Resuspension Buffer (R3) with RNase A and Lysis Indicator, then lyse the cells with Lysis Buffer (L7). The cell lysate turns blue to indicate complete lysis.

Add Precipitation Buffer (N3) to the lysate, which turns the lysate colorless, and clarify the lysate by passing it over a Lysate Clarification Column. The cleared lysate is then mixed with a proprietary Endotoxin Removal Buffer and then passed over a DNA-binding Column.

The negatively charged phosphates on the DNA backbone interact with the positive charges on the resin surface of the DNA-binding Column. Endotoxins, RNA, proteins, carbohydrates and other impurities are washed away using the Wash Buffer (W8) and the Endotoxin Removal Buffer. The plasmid DNA is then eluted under high salt conditions with Elution Buffer (E4). The eluted DNA is desalted and concentrated with an alcohol precipitation step.

PureLink™ Expi technology

The PureLink™ Expi technology is based on next generation anion-exchange chromatography. The technology uses a patented membrane resin to provide high plasmid DNA yields, low endotoxin levels, and reproducible performance.

The spacer arm with increased length provides improved DNA binding efficiency. The unique patented ion-exchange moiety provides high efficiency for separation of DNA from cellular contaminants including RNA.

Lysis Indicator

Lysis Indicator is a color indicator that provides visual confirmation of complete cell lysis and subsequent neutralization. Lysis Indicator can be added to the Resuspension buffer (R3) bottle before use, or alternatively, smaller amounts of Lysis Indicator can be added to aliquots of Resuspension Buffer (R3), for single plasmid preparations.

Lysis Indicator is added to Resuspension Buffer (R3) at a 1:1000 ratio (e.g., 6 µL of Lysis Indicator into 6 mL Buffer R3) to achieve the required working concentration. Make sufficient Lysis Indicator/ Resuspension Buffer (R3) working solution for the number of plasmid preparations being performed.

Product specifications

Table 1 Specifications of PureLink™ Expi Endotoxin-Free Plasmid Purification Kits

Specifications [1]	Maxiprep Kit
Starting *E. coli* culture volume at A_{600} 2.0–4.0	100 mL
Elution volume	15 mL
DNA yield [2]	up to 1.5 mg
Endotoxin levels	<0.1 EU/µg

[1] Specifications and results are based on high copy number plasmids.

[2] Varies with plasmid copy number, type, and size, as well as the volume and density of bacterial culture used.

Downstream applications

Plasmid DNA isolated using the PureLink™ Expi Endotoxin-Free Maxi Plasmid Purification Kit is suitable for use in a variety of downstream applications including:

- Mammalian cell transfection (including sensitive cell lines)
- *in vivo* transfection
- Protein expression (ExpiCHO and Expi293™ Expression Systems)
- DNA sequencing
- PCR cloning
- *in vitro* transcription

Kit contents and storage

The PureLink™ Expi Endotoxin-Free Plasmid Purification Kits are shipped at room temperature. Upon receipt, store all components at room temperature.

Table 2 Components of PureLink™ Expi Endotoxin-Free Plasmid Purification Kits

Component	Cat. No. A33073	Cat. No. A31217	Cat. No. A31231	Storage
	4 maxipreps	10 maxipreps	25 maxipreps	
Resuspension Buffer (R3)	72 mL	110 mL	275 mL	Room temperature
RNase A	650 µL	650 µL	1.5 mL	
Lysis Indicator	1 mL	1 mL	1 mL	
Lysis Buffer (L7)	72 mL	110 mL	275 mL	
Precipitation Buffer (N3)	72 mL	110 mL	275 mL	
Wash Buffer (W8)	330 mL	510 mL	2 × 510 mL	
Endotoxin Removal Buffer (ER)	25 mL	25 mL	62.5 mL	
Elution Buffer (E4)	132 mL	175 mL	440 mL	
TE Buffer (TE)	30 mL	30 mL	30 mL	
Endotoxin Free Water	21 mL	21 mL	2 × 21 mL	
Lysate Clarification Column (in 50-mL conical tube with white cap)	4 each	10 each	25 each	
Maxi DNA-binding Column	4 each	10 each	25 each	
50-mL conical tube with blue cap	4 each	10 each	25 each	
Endotoxin-free, 2-mL Tubes	4 each	10 each	25 each	

Required materials not supplied

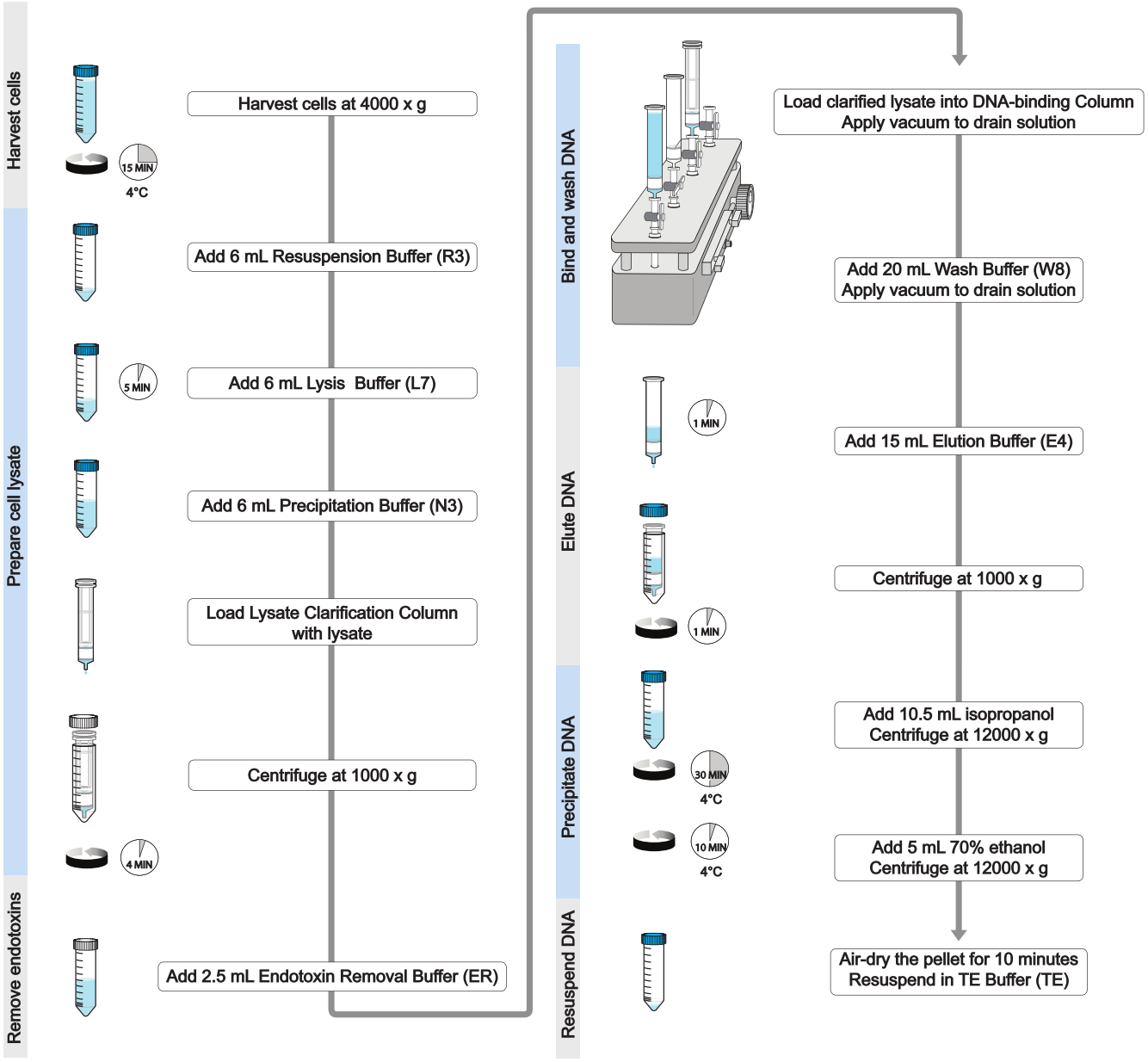
Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Equipment	
Centrifuge and swinging bucket rotor capable of reaching 1,000 × *g* at room temperature, and 4,000 × *g* at 4°C	MLS
Centrifuge and rotor capable of reaching >12,000 × *g* at 4°C	MLS
Vacuum manifold (e.g., the EveryPrep™ Universal Vacuum Manifold, Vac-Man™ Laboratory Vacuum Manifold, VM20 Vacuum Manifold, QIAvac 24 Plus, or equivalent), and vacuum source capable of generating a negative pressure of –600 to –800 mbar	MLS
Tubes, plates, and accessories	
(*Optional*) Sterile 50-mL centrifuge tubes	MLS
Sterile, endotoxin-free/pyrogen-free plastic pipette tips	MLS
Sterile, endotoxin-free/pyrogen-free centrifuge tubes (e.g. 30-mL or 50-mL tubes) for DNA precipitation	MLS
Reagents	
100% ethanol	MLS
100% isopropanol	MLS

Workflow

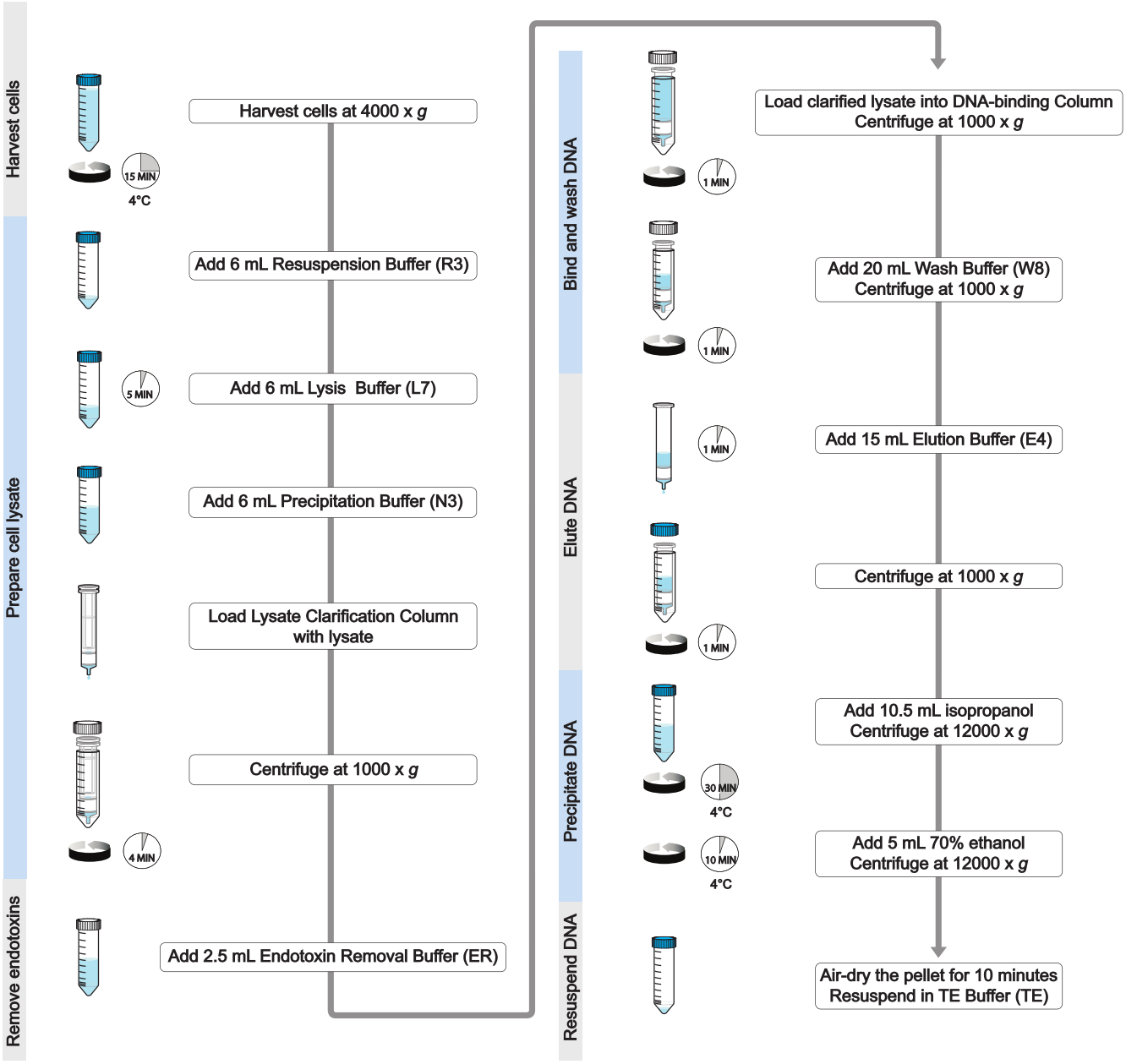
Vacuum protocol workflow

The following diagram is a simplified depiction of the maxiprep plasmid isolation procedure using a vacuum manifold. For detailed instructions on performing the protocol, see "Maxiprep procedure (vacuum manifold)" on page 15.



Centrifuge protocol workflow

The following diagram is a simplified depiction of the maxiprep plasmid isolation procedure using a centrifuge. For detailed instructions on performing the protocol, see "Maxiprep procedure (centrifuge only)" on page 17.



Methods

Procedural guidelines

Follow the recommendations below to obtain the best results:

- Ensure that no DNase or endotoxin is introduced into the sterile solutions supplied with the kits.
- Use sterile, endotoxin-free/pyrogen-free plastic pipette tips for transferring solutions from the elution step onwards to avoid contamination with endotoxin.
- Use the Endotoxin Free Water provided in the kit to prepare 70% Ethanol.
- Use endotoxin-free/pyrogen-free plasticware and reagents with purified plasmid DNA to avoid contamination with endotoxins.
- Resuspend the DNA pellet with the TE Buffer provided in the kit or endotoxin-free 10 mM Tris-HCl, pH 8.0.
- Store purified plasmid DNA in Endotoxin-free 2-mL Tubes provided in the kit, or endotoxin-free/pyrogen-free plastic tubes.

Before first use of the kit

Prepare Resuspension Buffer (R3)

1. Add RNase A to the Resuspension Buffer (R3) according to instructions on the bottle label. Mix well.
2. Mark the bottle label to indicate that RNase A is added.
3. (*Optional*) Add Lysis Indicator to Resuspension Buffer (R3) at a 1:1000 ratio.

   Note: Lysis Indicator may contain some particulate matter, but will function as expected. Resuspension Buffer (R3) turns slightly cloudy when Lysis Indicator is initially added.
   In addition, Lysis Indicator gradually precipitates after addition into Resuspension Buffer (R3). Shake Resuspension Buffer (R3) before use to resuspend Lysis Indicator particles. The remaining precipitate completely dissolves after addition of Lysis Buffer (L7).
4. Store Resuspension Buffer with RNase at 4°C.

Prepare 70% Ethanol

1. Add 50 mL of 100% ethanol to the bottle of Endotoxin Free Water provided in the kit. Mix well.
2. Label the bottle as 70% Ethanol.

Before each use of the kit

Guidelines for growing bacterial cultures

- Use bacterial cultures with a measured density at A_{600} of 2.0–4.0.
- Use a high copy-number plasmid cultured in LB media to obtain maximal yield and purity of plasmid DNA.
- Avoid using rich media for bacterial cultures. If using rich media is necessary, ensure that the recommended cell mass is not exceeded by optimizing growth time so that A_{600} <4.0, or by processing a smaller culture volume (<100 mL).
- Do not exceed the maximum recommended culture volumes given in the following table. Using larger culture volumes means increased cell mass, which will result in reduced yield and purity.

Table 3 Volumes of LB culture required for plasmid isolation according to plasmid copy number

Plasmid copy number	Maxiprep	Expected yield
High copy number plasmid	100 mL	0.5–1.5 mg
Low copy number plasmid	100–200 mL	0.1–0.5 mg

Grow bacterial cultures

1. Pick a single colony from a freshly streaked selective plate and inoculate a 1-mL starter culture of Luria-Bertani (LB) medium containing the appropriate selective antibiotic.

   Use a culture tube with a volume of at least 4 times the volume of the culture.

2. Incubate culture at 37°C for 8 hours with vigorous shaking (225–300 rpm).

3. Dilute the starter culture 1:1000 in LB medium containing the appropriate selective antibiotic in a flask with a volume of at least 4 times the volume of the culture.

   - High copy number plasmids—inoculate 100 mL of medium containing the appropriate selective antibiotic with 100 µL of starter culture.
   - Low copy number plasmids—inoculate 200 mL of medium containing the appropriate selective antibiotic with 200 µL of starter culture.

4. Incubate culture at 37°C for 12–16 hours with vigorous shaking (225–300 rpm).

5. Check the bacterial culture to ensure it has reached a cell density of approximately 2–4 × 10^9 cells/mL or an absorbance of 2.0–4.0 at 600 nm (A_{600}).

Check buffers for precipitate

1. Check Lysis Buffer (L7) for precipitate.

   If precipitate is present, warm the solution in a 37°C water bath for 5 minutes to redissolve any particulate matter.

2. Ensure that RNase A is added to the Resuspension Buffer (R3).

   If Lysis Indicator is added to Resuspension Buffer (R3), shake the bottle before use to resuspend any particulate matter (see “Lysis Indicator“ on page 6).

3. Ensure that 70% Ethanol is prepared with Endotoxin Free Water.

Maxiprep procedure (vacuum manifold)

Prepare cell lysate

1. Prepare overnight bacterial culture (A_{600} of 2.0–4.0) for plasmid isolation.
   - For **high copy number plasmids**, use ≤100 mL of an overnight LB culture.
   - For **low copy number plasmids**, use ≤100–200 mL of an overnight LB culture.
2. Harvest the cells by centrifuging the overnight LB culture at 4,000 × *g* for 15 minutes. Remove all medium.
3. Add 6 mL Resuspension Buffer (R3) with RNase A to the pellet and resuspend the cells until homogeneous by vortexing or pipetting. No cell clumps should be visible.

   Note: If using Lysis Indicator, shake Resuspension Buffer (R3) to resuspend particles.
4. Add 6 mL Lysis Buffer (L7). Mix gently by inverting the capped tube until the lysate mixture is homogeneous. **Do not vortex**. Incubate at room temperature for 5 minutes.

   Note: The presence of genomic DNA causes the mixture to be very viscous at this stage. Do not vortex the cell suspension because vortexing can cause genomic DNA shearing.
   If using Lysis Indicator, the solution turns completely blue.
5. Add 6 mL Precipitation Buffer (N3) and mix gently by inverting until the mixture is homogeneous. Do not vortex. A white, flocculent precipitate of proteins, cellular debris, genomic DNA, and detergent forms.

   Note: The neutralized lysate must be completely non-viscous, without any remnant of the viscous matter present after cell lysis.
   If using Lysis Indicator, the mixture turns completely colorless (i.e., the solution becomes clear, while the flocculent precipitate becomes white).
6. Pour the bacterial lysate from step 5 directly into the prepared Lysate Clarification Column (pre-inserted in a 50-mL conical tube with white cap). Centrifuge at 1,000 × *g* for 4 minutes (a swinging bucket rotor is recommended for best results).

   Note: For samples with high A_{600} value or large volume (>100 mL), pre-centrifugation at >5000 × *g* for 10 minutes is required to deplete the bulk of the flocculent material.
7. Remove and discard the Lysate Clarification Column from the 50-mL conical tube. Inspect the inside of the cap, and remove any flocculent material with a clean tissue.

   The 50-mL conical tube contains the clarified lysate with the plasmid DNA.
8. Add 2.5 mL Endotoxin Removal Buffer (ER) to the clarified lysate and mix by inverting the 50-mL conical tube (with white cap) 10 times.
9. Proceed to "Bind and wash DNA" on page 16.

Bind and wash DNA

1. Attach the DNA-binding Column to a vacuum manifold and load the clarified lysate from "Prepare cell lysate", step 8 onto the column. Apply vacuum to drain the solution.
2. Add 20 mL (one column volume) of Wash Buffer (W8) to the column and apply the vacuum to drain the solution.
   The binding column contains the DNA.
3. Proceed to "Elute DNA".

Elute DNA

IMPORTANT! Use sterile, endotoxin-free/pyrogen-free plasticware from this section onward to avoid contamination with endotoxins.

1. Inspect the DNA-binding Column, and remove any remaining liquid in the column or the tip with a clean tissue.
2. Place the binding column into an endotoxin-free 50-mL conical tube (with blue cap).
3. Add 15 mL of endotoxin-free Elution Buffer (E4) to the binding column.
   Note: Pre-heating Elution Buffer (E4) to 37–50°C can increase yield by ~20%.
4. Let the column stand for 1 minute without agitation.
5. Centrifuge at 1,000 × *g* for 1 minute (use a swinging bucket rotor for best results). Remove and discard the column.
   The 50-mL conical tube now contains the eluate with the purified DNA.
6. Proceed to "Precipitate DNA".

Precipitate DNA

Perform precipitation in the 50-mL tube in which the sample was eluted, or using your own endotoxin-free tube (e.g., 30-mL round bottom tube). Alternatively, for added convenience and speed, perform precipitation using a PureLink™ HiPure Precipitator (see page 27 for ordering information).

1. Add 0.7 volume of isopropanol to the eluate from "Elute DNA", step 5 and mix well (e.g, add 10.5 mL of isopropanol to 15 mL of eluate).
2. Centrifuge the tube at >12,000 × *g* for 30 minutes at 4°C.
3. Discard the supernatant. Wash the DNA pellet with 5 mL of 70% Ethanol.
4. Centrifuge at >12,000 × *g* for 10 minutes at 4°C and remove the 70% Ethanol.
5. Air-dry the DNA pellet for 10 minutes.
6. Resuspend the pellet in 1 mL of endotoxin-free TE Buffer (TE).

Store DNA

Store the purified DNA in Endotoxin-free 2-mL Tubes (provided in the kit) at 4°C for immediate use or make aliquots of the DNA and store at –20°C for long-term storage.

Maxiprep procedure (centrifuge only)

Prepare cell lysate

1. Prepare overnight bacterial culture (A_{600} of 2.0–4.0) for plasmid isolation.
   - For **high copy number plasmids**, use ≤100 mL of an overnight LB culture.
   - For **low copy number plasmids**, use ≤100–200 mL of an overnight LB culture.
2. Harvest the cells by centrifuging the overnight LB culture at 4,000 × *g* for 15 minutes. Remove all medium.
3. Add 6 mL Resuspension Buffer (R3) with RNase A to the pellet and resuspend the cells until homogeneous by vortexing or pipetting. No cell clumps should be visible.

   Note: If using Lysis Indicator, shake Resuspension Buffer (R3) to resuspend particles.
4. Add 6 mL Lysis Buffer (L7). Mix gently by inverting the capped tube until the lysate mixture is homogeneous. **Do not vortex**. Incubate at room temperature for 5 minutes.

   Note: The presence of genomic DNA causes the mixture to be very viscous at this stage. Do not vortex the cell suspension because vortexing can cause genomic DNA shearing.
   If using Lysis Indicator, the solution turns completely blue.
5. Add 6 mL Precipitation Buffer (N3) and mix gently by inverting until the mixture is homogeneous. Do not vortex. A white, flocculent precipitate of proteins, cellular debris, genomic DNA, and detergent forms.

   Note: The neutralized lysate must be completely non-viscous, without any remnant of the viscous matter present after cell lysis.
   If using Lysis Indicator, the mixture turns completely colorless (i.e., the solution becomes clear, while the flocculent precipitate becomes white).
6. Pour the bacterial lysate from step 5 directly into the prepared Lysate Clarification Column (pre-inserted in a 50-mL conical tube with white cap). Centrifuge in a swinging bucket rotor at 1,000 × *g* for 4 minutes.

   Note: For samples with high A_{600} value or large volume (>100 mL), pre-centrifugation at >5000 × *g* for 10 minutes is required to deplete the bulk of the flocculent material.
7. Remove and discard the Lysate Clarification Column from the 50-mL conical tube. Inspect the inside of the cap, and remove any flocculent material with a clean tissue.

   The 50-mL conical tube contains the clarified lysate with the plasmid DNA.
8. Add 2.5 mL Endotoxin Removal Buffer (ER) to the clarified lysate and mix by inverting the 50-mL conical tube (with white cap) 10 times.
9. Proceed to "Bind and wash DNA" on page 18.

Bind and wash DNA

1. Pour the clarified lysate from "Prepare cell lysate", step 8 onto the DNA-binding Column. Place the column into a 50-mL conical tube, and centrifuge in a swinging bucket rotor at 1,000 × *g* for 1 minute to drain the solution.

   Note: Reuse the 50-mL tube (with white cap) from "Prepare cell lysate", step 8, or use your own 50-mL tube

2. Discard the flow through, then add 20 mL (one column volume) of Wash Buffer (W8) to the Column. Centrifuge in a swinging bucket rotor at 1,000 × *g* for 1 minute to drain the solution.

   The binding column contains the DNA.

3. Proceed to "Elute DNA".

Elute DNA

IMPORTANT! Use sterile, endotoxin-free/pyrogen-free plasticware from this section onward to avoid contamination with endotoxins.

1. Inspect the DNA-binding Column, and remove any remaining liquid in the column or the tip with a clean tissue.

2. Place the binding column into an endotoxin-free 50-mL conical tube (with blue cap).

3. Add 15 mL of endotoxin-free Elution Buffer (E4) to the binding column.

   Note: Pre-heating Elution Buffer (E4) to 37–50°C can increase yield by ~20%.

4. Let the column stand for 1 minute without agitation.

5. Centrifuge at 1,000 × *g* for 1 minute in a swinging bucket rotor. Remove and discard the column.

   The 50-mL conical tube now contains the eluate with the purified DNA.

6. Proceed to "Precipitate DNA".

Precipitate DNA

Perform precipitation in the 50-mL tube in which the sample was eluted, or using your own endotoxin-free tube (e.g., 30-mL round bottom tube). Alternatively, for added convenience and speed, perform precipitation using a PureLink™ HiPure Precipitator (see page 27 for ordering information).

1. Add 0.7 volume of isopropanol to the eluate from "Elute DNA", step 5 and mix well (e.g, add 10.5 mL of isopropanol to 15 mL of eluate).

2. Centrifuge the tube at >12,000 × *g* for 30 minutes at 4°C.

3. Discard the supernatant. Wash the DNA pellet with 5 mL of 70% Ethanol.

4. Centrifuge at >12,000 × *g* for 10 minutes at 4°C and remove the 70% Ethanol.

5. Air-dry the DNA pellet for 10 minutes.

6. Resuspend the pellet in 1 mL of endotoxin-free TE Buffer (TE).

Store DNA

Store the purified DNA in Endotoxin-free 2-mL Tubes (provided in the kit) at 4°C for immediate use or make aliquots of the DNA and store at –20°C for long-term storage.

A

Troubleshooting

Observation	Possible cause	Recommended action
Low plasmid DNA yield	Temperature of Lysis Buffer (L7) or Elution Buffer (E4) is too low.	Store Lysis Buffer (L7) or Elution Buffer (E4) at room temperature.
	Low copy-number plasmid.	Increase the starting culture volume. See page "Guidelines for growing bacterial cultures" on page 13.
	Lysate is not at the proper pH or salt concentration for plasmid to bind the column.	Ensure that all culture medium is removed before resuspending cells.
		Make sure that the correct volume of Precipitation Buffer (N3) is added.
	No DNA precipitated (DNA is present in eluate aliquot but little or no DNA is recovered after precipitation).	Measure correctly the volume of eluate in each centrifugation tube and add exactly 0.7 volume of isopropanol.
		Centrifuge plasmid DNA at the appropriate speed, time, and temperature.
	Plasmid DNA pellet is over-dried.	Air-dry the DNA pellet. Do not dry the DNA pellet with a vacuum system.
	DNA-binding Column is overloaded.	Decrease the volume of the starting culture.
		Switch from maxiprep kit to megaprep kit (see "Accessory products" on page 27).
Lysate Clarification Column is clogged	Lysate Clarification Column is clogged by lysate.	Let the lysate stand in the column to allow the precipitate to float and form a layer on top of the lysate.
		Pre-centrifuge the lysate at 5,000 × *g* for 10 minutes to deplete the bulk of the flocculent material prior to passing the supernatant over the column.
High endotoxin levels	Insufficient mixing of Endotoxin Removal Buffer.	Invert tubes 10 times after addition of ER to ensure complete mixing.
Additional plasmid forms present	Plasmid DNA permanently denatured (band migrating faster than supercoiled DNA).	Incubate the lysate at room temperature for no longer than 5 minutes.
Genomic DNA contamination	Genomic DNA sheared during handling.	Gently invert tubes to mix after adding buffers L7 and N3. **Do not vortex** as it can shear genomic DNA.

Observation	Possible cause	Recommended action
RNA contamination	Lysate at improper pH, salt concentration, or temperature.	Carefully remove all medium before resuspending cells.
		Make sure not to add an excess of Precipitation Buffer (N3) when neutralizing the lysate.
		Do not warm the lysate above room temperature during centrifugation.
	Lysate left on column too long.	Once the lysate is loaded onto the column, avoid delays in processing.
	Lysate droplets remained on walls of column at elution.	Wash droplets of lysate from the walls of the column with the Wash Buffer (W8).
	RNase A digestion incomplete.	Verify RNase A is added to Resuspension Buffer (R3), and that it was stored at 4°C.
		Use recommended volume of buffer R3.

B

Determine yield and quality

Determine DNA yield

Measure the DNA concentration using UV absorbance at 260 nm or Qubit™ DNA Assay Kits.

Determine yield by NanoDrop™ Instrument

Measure UV absorbance using 1–2 µL of plasmid DNA sample with a NanoDrop™ spectrophotometer (see page 28 for ordering information)

Determine yield by spectro-photometer

Perform measurement of UV absorbance on a standard spectrophotometer as follows:

1. Prepare a dilution of the plasmid DNA sample with the TE Buffer provided in the kit or endotoxin-free 10 mM Tris-HCl, pH 8.0. Mix well.
2. Measure the absorbance at 260 nm (A_{260}) of the dilution (using a cuvette with an optical path length of 1 cm) in a spectrophotometer blanked against TE Buffer/10 mM Tris-HCl, pH 8.0.
3. Calculate the concentration of DNA using the formula:
   DNA (µg/mL) = A_{260} × 50 × dilution factor

   Note: For DNA, A_{260} = 1 for a 50 µg/mL solution measured in a cuvette with an optical path length of 1 cm.

Determine yield by Qubit™ DNA Assay Kit

Use 1–20 µL of plasmid DNA sample to determine yield using Qubit™ DNA Assay Kits (see page "Accessory products" on page 27 for ordering information)

The assay is designed for taking measurements using the Qubit™ 3.0 Fluorometer.

Determine DNA quality

Typically, DNA isolated using the PureLink™ Expi Endotoxin-Free Maxi Plasmid Purification Kit has an A_{260}/A_{280} ratio >1.8 when samples are diluted in Tris-HCl pH 8.0, indicating that the DNA is substantially clean of proteins that could interfere with downstream applications.

Confirm absence of contaminating RNA by agarose gel electrophoresis or by performing a Qubit™ RNA assay.

Plasmid DNA isolated with the PureLink™ Expi Endotoxin-Free Maxi Plasmid Purification Kit is mainly in supercoiled form and appears as one major band on agarose gels.

C

Expected results

Summary of expected results

The summary of results using the PureLink™ Expi Endotoxin-Free Maxi Plasmid Purification Kit is listed in the table below.

Results for:	Maxiprep kit
Processing time	90 minutes
Plasmid DNA yield [1]	Up to 1.5 mg
Endotoxin levels	<0.1 EU/µg
Sequencing	Successful
Restriction enzyme digestion	Successful
Transfection (sensitive cell lines)	Successful
Expression (ExpiCHO and Expi293 Expression Systems)	Successful

[1] DNA yield depends on plasmid copy number and type, bacterial strain, and growth conditions.

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Accessory products

Accessory products

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Amount	Source
PureLink™ Expi Endotoxin-Free Mega Plasmid Purification Kit	4 preps	Cat. No. A31232
PureLink™ Expi Endotoxin-Free Giga Plasmid Purification Kit	2 preps	Cat. No. A31233
PureLink™ Expi Endotoxin-Free Buffer Set	1 kit	Cat. No. A33074
PureLink™ PCR Purification Kit	50 preps	Cat. No. K3100-01
PureLink™ Quick Gel Extraction Kit	50 preps	Cat. No. K2100-12
Expi293™ Expression System Kit	1 kit	Cat. No. A14635
ExpiCHO™ Expression System Kit	1 kit	Cat. No. A29133
Qubit™ 3.0 Fluorometer	1 each	Cat. No. Q33216
Qubit™ dsDNA BR Assay Kit	500 assays	Cat. No. Q32853
Luria Broth Base (Miller's LB Broth Base), powder	500 g 2.5 kg	Cat. No. 12795-027 Cat. No. 12795-084
Ampicillin Sodium Salt, irradiated	200 mg	Cat. No. 11593-027
EveryPrep™ Universal Vacuum Manifold	1 each	Cat. No. K211101
PureLink™ HiPure Precipitator Module	10 precipitators 25 precipitators	Cat. No. K2100-21 Cat. No. K2100-22
PureLink™ HiPure Plasmid FP (Filter and Precipitator) Maxiprep Kit	10 preps 25 preps	Cat. No. K2100-26 Cat. No. K2100-27
PureLink™ HiPure Plasmid Filter Midiprep Kit	25 preps 50 preps	Cat. No. K2100-14 Cat. No. K2100-15
PureLink™ HiPure Plasmid Filter Maxiprep Kit	10 preps 25 preps	Cat. No. K2100-16 Cat. No. K2100-17

E-Gel™ agarose gels and DNA ladders

E-Gel™ Agarose Gels are bufferless pre-cast agarose gels with a variety of different agarose percentages and well formats designed for fast, convenient electrophoresis of DNA samples.

To find DNA ladders available for sizing DNA, visit **thermofisher.com** or contact **Technical Support** (page 29) for more details on these products.

NanoDrop™ instruments

Quantify and qualify your plasmid DNA samples and get full-spectral data in seconds using the NanoDrop™ spectrophotometer. The NanoDrop™ spectra data helps identify contaminants and calculates corrected concentrations. Visit **nanodrop.com** for more information on NanoDrop™ instruments.

F Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.