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PureLink™ Expi Endotoxin-Free Mega and Giga Plasmid Purification Kits

USER GUIDE

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

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ThermoFisher SCIENTIFIC

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Product information

Product description

The PureLink™ Expi Endotoxin-Free Mega and Giga Plasmid Purification Kits are used to isolate 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 Cartridges utilize a unique, proprietary, enhanced anion exchange membrane that enables rapid, large scale purification of endotoxin-free plasmid DNA, in as little as 120 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 resin used in PureLink™ HiPure 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 PureLink™ Expi Endotoxin-Free Mega and Giga Plasmid Purification Kits.

Advantages of the PureLink™ Expi Endotoxin-Free Plasmid Purification Kits

- **High Yield**
  Isolate up to 5 mg of high quality endotoxin-free plasmid DNA from 0.5–1 L of bacterial culture with the PureLink™ Expi Endotoxin-Free Mega Plasmid Purification Kit.
  Isolate up to 15 mg of high quality endotoxin-free plasmid DNA from 2.5–5 L of bacterial culture with the PureLink™ Expi Endotoxin-Free Giga Plasmid Purification Kit.
- **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**

  A unique vacuum assisted protocol with advanced membrane technology enables the fastest anion exchange purifaction, and allows a typical mega or giga plasmid isolation to be performed in as little as 120 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 vacuum-assisted clarification filter. The cleared lysate is then mixed with a proprietary Endotoxin Removal Buffer and then passed over a vacuum-assisted DNA-Binding Cartridge.

The negatively charged phosphates on the DNA backbone interact with the positive charges on the resin surface of the DNA-Binding Cartridge. 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.

HiPure technology

The HiPure 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., 50 µL of Lysis Indicator into 50 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]	Megaprep Kit	Gigaprep Kit
Starting *E. coli* culture volume at OD 2.0–4.0	500 mL	2.5 L
Binding capacity	5 mg	15 mg
Expi Lysate Filtration Cartridge reservoir capacity	230 mL	450 mL
Expi DNA-Binding Cartridge reservoir capacity	200 mL	400 mL
Elution volume	50 mL	100 mL
DNA yield [2]	up to 5 mg	up to 15 mg
Endotoxin levels	<0.1 EU/µg	<0.1 EU/µg

[1] Specifications and results are based on high copy number plasmids.
[2] Varies with plasmid copy number, type and size, and volume of bacterial culture used.

Downstream applications

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

- Mammalian cell transfection (including sensitive cell lines)
- *in vivo* transfection
- 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.

- The PureLink™ Expi Endotoxin-Free Mega Plasmid Purification Kit contains sufficient components to perform four megapreps.
- The PureLink™ Expi Endotoxin-Free Giga Plasmid Purification Kit contains sufficient components to perform two gigapreps.

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

Component	Cat. No. A31232	Cat. No. A31233	Storage
	Megaprep Kit	Gigaprep Kit	
Resuspension Buffer (R3)	220 mL	275 mL	Room temperature
RNase A	1.5 mL	1.5 mL	
Lysis Indicator	300 µL	300 µL	
Lysis Buffer (L7)	220 mL	275 mL	
Precipitation Buffer (N3)	220 mL	275 mL	
Equilibration Buffer (EQ1)	440 mL	440 mL	
Wash Buffer (W8)	4 × 440 mL	3 × 440 mL	
Endotoxin Removal Buffer (ER)	62.5 mL	62.5 mL	
Elution Buffer (E4)	440 mL	440 mL	
TE Buffer (TE)	30 mL	30 mL	
Endotoxin Free Water	21 mL	21 mL	
Expi Megaprep Lysate Filtration Cartridge	4 each	—	
Expi Gigaprep Lysate Filtration Cartridge	—	2 each	
Expi Megaprep DNA-Binding Cartridge	4 each	—	
Expi Gigaprep DNA-Binding Cartridge	—	2 each	
Pyrogen-free, 2-mL Tubes	4 each	4 each	

Buffer composition

Table 3 Composition of buffers in the PureLink™ Expi Endotoxin-Free Plasmid Purification Kits

Buffer	Composition
Resuspension Buffer (R3)	50 mM Tris-HCl, pH 8.0 10 mM EDTA
RNase A	20 mg/mL in 10 mM sodium acetate, pH 5.2
Lysis Buffer (L7)	0.2 M NaOH 1% (w/v) SDS
Precipitation Buffer (N3)	3.1 M potassium acetate, pH 5.5
Equilibration Buffer (EQ1)	0.1 M sodium acetate, pH 5.0 0.6 M NaCl 0.15% (v/v) Triton™ X-100
Wash Buffer (W8)	0.1 M sodium acetate, pH 5.0 825 mM NaCl
Elution Buffer (E4)	100 mM Tris-HCl, pH 8.5 1.25 M NaCl Nuclease-free water (<0.25 EU/mL)
TE Buffer (TE)	10 mM Tris-HCl, pH 8.0 0.1 mM EDTA Nuclease-free water (<0.25 EU/mL)
Endotoxin Free Water	Nuclease-free water (<0.25 EU/mL)

Required materials not supplied

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

Item	Source
Equipment	
Centrifuge and rotor capable of reaching >12,000 × *g* at 4°C	MLS
Buckets, rotor, and centrifuge for harvesting cells	MLS
Vacuum source capable of generating a negative pressure of –600 to –800 mbar	MLS
Tubes, plates, and accessories	
Sterile centrifuge tubes (e.g. 30-mL or 50-mL tubes)	MLS
Sterile container for transferring the lysate	MLS
Sterile spatula	MLS
1000-mL Stericup™ Receiver Flask with 45-mm thread neck (Millipore Cat. No. SC00B10RE) or a sterile 1-L glass bottle	MLS
250-mL Stericup™ Receiver Flask with 45-mm thread neck (Millipore Cat. No. SC00B10RE) or a sterile, **endotoxin-free** glass bottle for collection of DNA eluate	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
Isopropanol	MLS

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.
- Prepare 70% Ethanol with the Endotoxin Free Water provided in the kit.
- Save aliquots as recommended during the purification procedure. The aliquots allow you to troubleshoot if there are problems with the plasmid isolation procedure (see "Results from collected aliquots" on page 20).
- Maintain a sterile environment when handling DNA to avoid any contamination with DNase.
- Use endotoxin-free/pyrogen-free plasticware or 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 Pyrogen-free 2-mL Tubes provided in the kit, or endotoxin-free/pyrogen-free plastic tubes.
- Use endotoxin-free glassware used from the elution step onwards to avoid contamination with endotoxins.
- Bake glassware overnight at 180°C in a dry heat oven dedicated for depyrogenation to destroy endotoxins.

Guidelines for resuspending DNA

- Air-dry the DNA pellet instead of using a vacuum, especially if the DNA is of high molecular weight.
- Ensure that the pH of the buffer used to redissolve the DNA pellet is ≥8.0. DNA dissolves best under alkaline conditions; it does not easily dissolve in acidic buffers.
- Allow more time for the DNA pellet to go into solution.
- If the DNA pellet is not going into solution, warm the solution slightly.
- If the solution containing the DNA pellet becomes highly viscous, increase the volume of buffer used for dissolving the DNA.

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

Determine bacterial culture volume

Use a high copy-number plasmid to obtain a good yield of plasmid DNA. High copy number plasmids typically yield 2–6 µg DNA/mL LB culture with an A_{600} of 2.0.

Typical yields from low copy number plasmids are highly dependent upon culture conditions and vector/host strain combinations. If you are using a low copy-number plasmid, use a higher volume of LB culture, as directed in the following table.

Table 4 Volumes of LB culture required for plasmid isolation according to plasmid copy number

Plasmid copy number	Megaprep	Gigaprep
High copy number plasmid	500 mL	2.5 L
Low copy number plasmid	2.5 L	5 L

Grow bacterial cultures

Plasmid isolation is performed on stationary phase bacterial cultures with a cell density of approximately 10^9 cells/mL or an absorbance of 2.0–4.0 at 600 nm (A_{600}).

Grow transformed *E. coli* cells overnight in Luria-Bertani (LB) medium with the appropriate antibiotic.

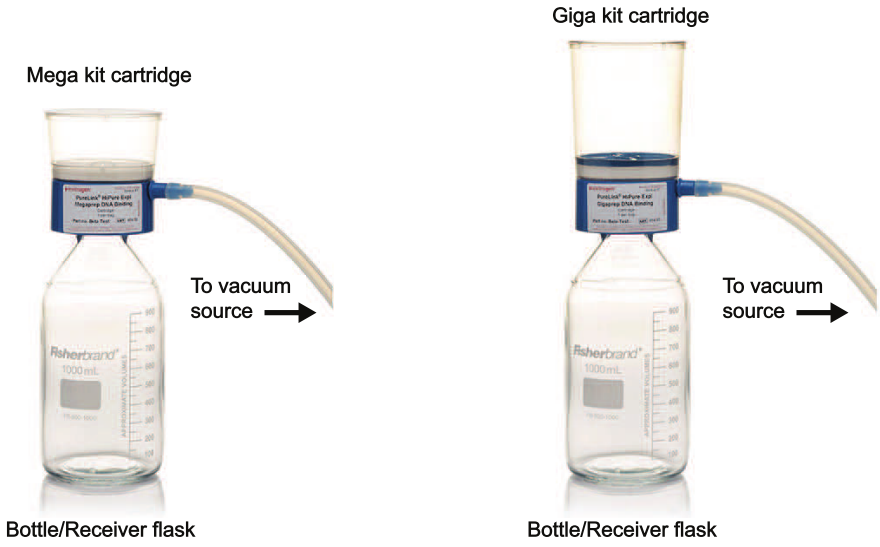
Check buffers

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.

Set up the Lysate Filtration/DNA Binding Cartridge

1. Attach the Megaprep or Gigaprep Lysate Filtration/DNA Binding Cartridge onto a clean Stericup™ Receiver Flask or sterile bottle with 45-mm thread neck.

   Note: Do not over-tighten the filtration cartridge on the bottleneck or the plastic filtration cartridge may crack.
2. Connect the vacuum source to the tubing connector of the filtration cartridge. Examples of the setups are shown in the following figure.



Megaprep procedure

The PureLink™ Expi Endotoxin-Free Mega Plasmid Purification Kit procedure can be completed in 120 minutes using 500 mL of overnight culture containing high copy number plasmids, or 2.5 L of overnight culture containing low copy number plasmids.

For instructions on performing the PureLink™ Expi Endotoxin-Free Giga Plasmid Purification Kit procedure, see page 17.

Prepare cell lysate

1. Prepare overnight bacterial culture for plasmid isolation.
   - For **high copy number plasmids**, use up to 500 mL of an overnight LB culture.
   - For **low copy number plasmids**, use up to 2.5 L of an overnight LB culture.
2. Harvest the cells by centrifuging the overnight LB culture at 4000 × *g* for 15 minutes. Remove all medium.
3. Add 50 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 50 mL Lysis Buffer (L7). Mix gently by inverting the capped bottle 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 may cause genomic DNA shearing.
5. Add 50 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 (step 4).
   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 Filtration Cartridge (see page 13). Incubate the cartridge at room temperature for **at least 2 minutes** without agitation.

   IMPORTANT! To prevent clogging and ensure efficient filtration, let the lysate stand for at least 2 minutes in the cartridge to allow the precipitate to float to the surface of the lysate.

7. Ensure that the vacuum source is connected to the tubing connector of the filtration cartridge and apply the vacuum (−600 to −800 mbar). Collect the clear flow-through into the flask. Keep the vacuum on until all of the liquid has drained, then turn off the vacuum source.

   Note: An unclogged column filters 150 mL of lysate in 1–2 minutes when vacuum is applied.

8. Add 50 mL Wash Buffer (W8) to the filtration cartridge and gently stir the precipitate with a sterile spatula.

   Note: Gentle agitation of the precipitate improves the flow of liquid through the filter unit.

9. Apply the vacuum until all of the liquid has drained from the filtration cartridge.
   The receiving flask contains the clarified lysate with the plasmid DNA.

10. Remove the filtration cartridge, and discard as biohazardous waste.

11. Add 12.5 mL Endotoxin Removal Buffer (ER) to the clarified lysate and mix by inverting the bottle 10 times.

12. Proceed to "Bind and wash DNA".

Bind and wash DNA

1. Attach a DNA-Binding Cartridge onto a 1000-mL Stericup™ Receiver Flask or equivalent.

2. Add 100 mL Equilibration Buffer (EQ1) to the binding cartridge. Apply the vacuum through tubing connected to the binding cartridge connector and keep the vacuum on until all of the liquid has drained from the binding cartridge. Then, turn off the vacuum and discard the flow-through.

3. Load the filtered lysate from "Prepare cell lysate", step 11 into the equilibrated Megaprep DNA-Binding Cartridge and apply vacuum until all of the lysate has passed through the binding cartridge.
   (*Optional*) Save a 100-µL aliquot of the flow-through for further analysis.

4. Add 175 mL Wash Buffer (W8) to the cartridge and apply the vacuum until all of the liquid has drained from the cartridge. Turn off the vacuum.
   (*Optional*) Save a 100-µL aliquot of the flow-through for further analysis.

5. **Repeat** step 4, once. Discard the flow-through.
   The binding cartridge contains the DNA.

6. Proceed to "Elute DNA" on page 16.

Elute DNA

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

1. Remove the DNA-Binding Cartridge (containing the DNA) from the 1000-mL Stericup™ Receiver Flask and attach the binding cartridge onto a clean, sterile 250-mL Stericup™ Receiver Flask or equivalent.
2. Add 50 mL of endotoxin-free Elution Buffer (E4) to the binding cartridge.

   Note: If a smaller elution volume is desired, elute with as little as 35 mL of Elution Buffer (E4).
   Pre-heating Elution Buffer (E4) to 37–50°C can increase yield by ~20%.
3. Apply vacuum through the tubing attached to the binding cartridge connector until approximately 10–20 mL of Elution Buffer (E4) has passed through the cartridge. Then, turn off the vacuum.
4. Let the cartridge stand for 1 minute without agitation.
5. Apply the vacuum again until all of the Elution Buffer (E4) has drained from the binding cartridge into the Stericup™ Receiver Flask.
   The 250-mL receiver flask now contains the eluate with the purified DNA.
   (*Optional*) Save a 100-µL aliquot of the flow-through for further analysis.
6. Proceed to "Precipitate DNA".

Precipitate DNA

1. Transfer all of the eluate from "Elute DNA" on page 16, step 5 into clean centrifuge tubes.

   Note: The number of tubes depends on the elution volume and the capacity of the centrifuge tubes.
   Do not fill centrifuge tubes to the top. Ensure that sufficient space remains for addition of isopropanol (e.g., add a maximum 17 mL of eluate to a 30-mL tube).
2. Add 0.7 volume isopropanol per volume of eluate and mix well (e.g, add 12 mL of isopropanol to 17 mL of eluate).
3. Centrifuge the tubes >12,000 × *g* for 30 minutes at 4°C.
4. Discard the supernatant. Wash the DNA pellets with 70% Ethanol.

   Note: The total volume used for washing all of the pellets should not exceed 15 mL (e.g., for three tubes, use no more than 5 mL of 70% Ethanol for each tube).
5. Centrifuge at >12,000 × *g* for 10 minutes at 4°C and remove the 70% Ethanol.
6. Air-dry the DNA pellets for 10 minutes.
7. Resuspend the pellets in a total of 1–2 mL of endotoxin-free TE Buffer (TE). See "Guidelines for resuspending DNA" on page 11 for details.

Store DNA

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

Gigaprep procedure

The PureLink™ Expi Endotoxin-Free Giga Plasmid Purification Kit procedure can be completed in 120 minutes using 2.5 L of overnight culture containing high copy number plasmids, or 5 L of overnight culture containing low copy number plasmids.

For instructions on performing the PureLink™ Expi Endotoxin-Free Mega Plasmid Purification Kit procedure, see page 14.

Prepare cell lysate

1. Prepare overnight bacterial culture for plasmid isolation.
   - For **high copy number plasmids**, use up to 2.5 L of an overnight LB culture.
   - For **low copy number plasmids**, use up to 5 L of an overnight LB culture.
2. Harvest the cells by centrifuging the overnight LB culture at 4000 × *g* for 15 minutes. Remove all medium.
3. Add 125 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 125 mL Lysis Buffer (L7). Mix gently by inverting the capped bottle 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 may cause genomic DNA shearing.
5. Add 125 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 (step 4).
   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 Filtration Cartridge (see page 13). Incubate the cartridge at room temperature for **at least 5 minutes** without agitation.

   IMPORTANT! To prevent clogging and ensure efficient filtration, let the lysate stand for at least 5 minutes in the cartridge to allow the precipitate to float to the surface of the lysate.

7. Ensure that the vacuum source is connected to the tubing connector of the filtration cartridge and apply the vacuum (−600 to −800 mbar). Collect the clear flow-through into the flask. Keep the vacuum on until all of the liquid has drained, then turn off the vacuum source.

   Note: An unclogged column filters 375 mL of lysate in 2–4 minutes when vacuum is applied.

8. Add 50 mL Wash Buffer (W8) to the filtration cartridge and gently stir the precipitate with a sterile spatula.

   Note: Gentle agitation of the precipitate improves the flow of liquid through the filter unit.

9. Apply the vacuum until all of the liquid has drained from the filtration cartridge.

   The receiving flask contains the clarified lysate with the plasmid DNA.

10. Remove the filtration cartridge, and discard as biohazardous waste.

11. Add 30 mL Endotoxin Removal Buffer (ER) to the clarified lysate and mix by inverting the bottle 10 times.

12. Proceed to "Bind and wash DNA".

Bind and wash DNA

1. Attach a DNA-Binding Cartridge onto a 1000-mL Stericup™ Receiver Flask or equivalent.

2. Add 200 mL Equilibration Buffer (EQ1) to the binding cartridge. Apply the vacuum through tubing connected to the binding cartridge connector and keep the vacuum on until all of the liquid has drained from the binding cartridge. Then, turn off the vacuum and discard the flow-through.

3. Load the filtered lysate from "Prepare cell lysate", step 11 into the equilibrated Gigaprep DNA-Binding Cartridge and apply vacuum until all of the lysate has passed through the binding cartridge.

   (*Optional*) Save a 100-µL aliquot of the flow-through for further analysis.

4. Add 275 mL Wash Buffer (W8) to the cartridge and apply the vacuum until all of the liquid has drained from the cartridge. Turn off the vacuum.

   (*Optional*) Save a 100-µL aliquot of the flow-through for further analysis.

5. **Repeat** step 4, once. Discard the flow-through.

   The binding cartridge contains the DNA.

6. Proceed to "Elute DNA" on page 19.

Elute DNA

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

1. Remove the DNA-Binding Cartridge (containing the DNA) from the 1000-mL Stericup™ Receiver Flask and attach the binding cartridge onto a clean, sterile 250-mL Stericup™ Receiver Flask or equivalent.
2. Add 100 mL of endotoxin-free Elution Buffer (E4) to the binding cartridge.

   Note: If a smaller elution volume is desired, elute with as little as 70 mL of Elution Buffer (E4).

   Pre-heating Elution Buffer (E4) to 37–50°C can increase yield by ~20%.
3. Apply vacuum through the tubing attached to the binding cartridge connector until approximately 30–40 mL of Elution Buffer (E4) has passed through the cartridge. Then, turn off the vacuum.
4. Let the cartridge stand for 1 minute without agitation.
5. Apply the vacuum again until all of the Elution Buffer (E4) has drained from the binding cartridge into the Stericup™ Receiver Flask.

   The 250-mL receiver flask now contains the eluate with the purified DNA.

   (*Optional*) Save a 100-µL aliquot of the flow-through for further analysis.
6. Proceed to "Precipitate DNA".

Precipitate DNA

1. Transfer all of the eluate from "Elute DNA" on page 19, step 5 into clean centrifuge tubes.

   Note: The number of tubes depends on the elution volume and the capacity of the centrifuge tubes.

   Do not fill centrifuge tubes to the top. Ensure that sufficient space remains for addition of isopropanol (e.g., add a maximum 17 mL of eluate to a 30-mL tube).
2. Add 0.7 volume isopropanol per volume of eluate and mix well (e.g, add 12 mL of isopropanol to 17 mL of eluate).
3. Centrifuge the tubes >12,000 × *g* for 30 minutes at 4°C.
4. Discard the supernatant. Wash the DNA pellets with 70% Ethanol.

   Note: The total volume used for washing all of the pellets should not exceed 30 mL (e.g., for six tubes, use no more than 5 mL of 70% Ethanol for each tube).
5. Centrifuge at >12,000 × *g* for 10 minutes at 4°C and remove the 70% Ethanol.
6. Air-dry the DNA pellets for 10 minutes.
7. Resuspend the pellets in a total of 3–4 mL of endotoxin-free TE Buffer (TE). See "Guidelines for resuspending DNA" on page 11 for details.

Store DNA

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

A Troubleshooting

Results from collected aliquots

If problems arise during the procedure, analyze the aliquots harvested during the procedure on a 1% agarose gel to determine the presence of DNA in the aliquots. The table below lists the normal and abnormal results and probable cause.

Aliquot	Expected result	Abnormal result	Cause of abnormal result
DNA-Binding Cartridge flow-through	No DNA	DNA	• Lysate at improper pH or salt concentration for plasmid DNA to bind column. • Cartridge overloaded. • DNA-Binding Cartridge damaged.
DNA-Binding Cartridge wash	No DNA	DNA	DNA-Binding Cartridge damaged.
DNA-Binding Cartridge eluate	DNA	No DNA	Incorrect pH or salt concentration to release plasmid DNA from column. **Note:** Elute with warm (37°C to 50°C) Elution Buffer (E4).

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 12
	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.
	DNA-Binding Cartridge is damaged.	Do not damage the cartridge during the procedure by over-tightening the cartridge or dropping it.
	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 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 Cartridge is overloaded.	Decrease the volume of the starting culture.
		Switch to gigaprep protocol if currently using megaprep protocol.
Lysate Filtration Cartridge is clogged	Lysate Filtration Cartridge is clogged by lysate.	Let the lysate stand in the cartridge to allow the precipitate to float and form a layer on top of the lysate.
		Centrifuge the lysate prior to passing the supernatant over the cartridge.
High endotoxin levels	Insufficient mixing of Endotoxin Removal Buffer.	Add Endotoxin Removal Buffer to clarified lysate before DNA binding step.
		Invert bottles 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.

Determine yield and quality

After isolating DNA, determine the quantity and quality of the purified DNA.

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 30 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

Measure 1–20 µL of plasmid DNA sample using Qubit™ DNA Assay Kits (see page 29 for ordering information)

The assay is designed for taking measurements using the Qubit™ 2.0 Fluorometer.

Determine DNA quality

Typically, DNA isolated using PureLink™ Expi Endotoxin-Free Mega and Giga Plasmid Purification Kits 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 Mega and Giga Plasmid Purification Kits 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 Mega and Giga Plasmid Purification Kits is listed in the table below.

Note: DNA yield depends on plasmid copy number and type, bacterial strain, and growth conditions.

Results for:	Megaprep kit	Gigaprep kit
Processing time	120 minutes	120 minutes
Plasmid DNA yield [1]	Up to 5 mg	Up to 15 mg
Endotoxin levels	<0.1 EU/µg	<0.1 EU/µg
Sequencing	Successful	Successful
Restriction enzyme digestion	Successful	Successful
Transfection (sensitive cell lines)	Successful	Successful
Expression (ExpiCHO and Expi 293 Expression Systems)	Successful	Successful

[1] Yields can vary depending on the bacterial strain, mass of the bacterial pellet, and plasmid.

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, and 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. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may 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 (**www.fisherscientific.com**) or other major laboratory supplier.

Item	Amount	Source
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
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
PureLink™ HiPure Plasmid FP (Filter and Precipitator) Maxiprep Kit	10 preps 25 preps	Cat. No. K2100-26 Cat. No. K2100-27
Qubit™ 2.0 Fluorometer	1 each	Cat. No. Q32866
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

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 31) 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.

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
- 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**.