PureLink™ Expi Endotoxin-Free Maxi Plasmid Purification Kit

invitrogen

QUICK REFERENCE

Publication No. MAN0016161 **Rev** B.0

Package Contents

Catalog Numbers	Amount:
A33073	4 preps
A31217	10 preps
A31231	25 preps

Storage Conditions

- Store all components at room temperature.

Required Materials

- Compatible vacuum manifold with vacuum source capable of –600 to –800 mbar (vacuum protocol only)
- 100% isopropanol
- 100% ethanol
- Centrifuges with swinging bucket rotors capable of 1,000 × *g* at room temperature, and 4,000 × *g* at 4°C; high speed centrifuge capable of >12, 000 × *g* at 4°C

Timing

Bacterial culture: overnight

Purification: 90 minutes

Selection Guide

Go online to view related products:

PureLink™ Nucleic Acid Purification Kits

Expi293™ and ExpiCHO™ Expression Systems

Product Description

- The PureLink™ Expi Endotoxin-Free Maxi Plasmid Purification Kit enables isolation of highly pure, endotoxin-free (<0.1 EU/µg) advanced transfection grade plasmid DNA for use in your most sensitive downstream applications.
- Isolate up to 1.5 mg of high quality, endotoxin-free ultrapure plasmid DNA from 100–200 mL of bacterial culture.
- Endotoxin-free (<0.1 EU/µg) DNA with ultra-low levels of RNA, gDNA or protein contamination that is ideal for mammalian cell transfection or *in vivo* experiments.
- Lysis Indicator turns blue during lysis and helps to ensure complete lysis and subsequent neutralization.

Important Guidelines

- Add Lysis Indicator to Resuspension Buffer (R3) at a 1:1000 ratio if desired.
- If precipitate is observed in the Lysis Buffer (L7), warm the buffer in a 37°C water bath until the solution clears. Swirl contents gently to resuspend.
- Grow transformed *E. coli* in LB medium. Use 100 mL (high copy number plasmid) or 200 mL (low copy number plasmid) of an overnight culture.
- Perform protocol (step 6 onward) using endotoxin-free/pyrogen free components.
- Do not over-dry DNA. If the DNA pellet is difficult to resuspend, allow the pellet to incubate in TE Buffer for a longer period of time.

Online Resources

Visit our product page for additional information and protocols. For support, visit **thermofisher.com/support**.

For Research Use Only. Not for use in diagnostic procedures.

Maxiprep plasmid isolation protocol (centrifuge)

Before first use of the kit, add RNase A to the Resuspension Buffer (R3) according to the label on the bottle (Mark the label to indicate that RNase A is added. Store at 4°C). Prepare endotoxin-free 70% ethanol by adding 100% ethanol to endotoxin-free water.

	Steps	Procedure Details
1	**Harvest**	Sediment cells by centrifugation at 4,000 × *g* for 15 min at 4°C. Discard all medium.
2	**Resuspend**	Add 6 mL Resuspension Buffer (R3) with RNase A to the cell pellet and resuspend the pellet until it is homogeneous.
3	**Lyse**	Add 6 mL Lysis Buffer (L7). Mix gently by inverting the capped tube until the mixture is homogeneous. Do not vortex. Incubate at room temperature for 5 minutes. **Note**: If using Lysis Indicator, the solution turns blue.
4	**Precipitate**	Add 6 mL Precipitation Buffer (N3). Mix immediately by inverting the tube until the mixture is homogeneous. Do not vortex. **Note**: If using Lysis Indicator, the solution turns completely colorless.
5	**Clarify**	Pour the lysate into a **Lysate Clarification Column** (pre-inserted in a 50-mL conical tube with white cap). Centrifuge at 1,000 × *g* for 4 minutes.
6	**Remove Endotoxins**	Remove and discard the Lysate Clarification Column. 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.
7	**Bind**	Pour the **clarified lysate** (from step 6) into a **DNA-binding Column**. Place the column into the 50-mL conical tube (with white cap) from step 6, and Centrifuge in a swinging bucket rotor at 1,000 × *g* for 1 minute to drain the solution.
8	**Wash**	Discard flow through, then add 20 mL (one column volume) of Wash Buffer (W8) to the DNA-binding Column and centrifuge in a swinging bucket rotor at 1,000 × *g* for 1 minute to drain the solution.
9	**Elute**	Place the DNA-binding Column into an endotoxin-free 50-mL conical tube (with blue cap). Add 15 mL Elution Buffer (E4) to the column and incubate for 1 minute. Centrifuge at 1,000 × *g* for 1 minute. Remove and discard the column.
10	**Precipitate and Wash**	Add 10.5 mL (0.7 volume) of isopropanol to the eluate. Mix well. Centrifuge at >12,000 × *g* for 30 minutes at 4°C. Remove and discard the supernatant. Wash the DNA pellet with 5 mL 70% ethanol. Centrifuge at >12,000 × *g* for 10 minutes at 4°C. Remove the supernatant.
11	**Resuspend**	Air-dry the pellet for 10 minutes, then resuspend the purified plasmid DNA in TE Buffer (TE). Store plasmid DNA in endotoxin-free tubes at –20°C.

Maxiprep plasmid isolation protocol (vacuum)

Before first use of the kit, add RNase A to the Resuspension Buffer (R3) according to the label on the bottle (Mark the label to indicate that RNase A is added. Store at 4°C). Prepare endotoxin-free 70% ethanol by adding 100% ethanol to endotoxin-free water.

	Steps	Procedure Details
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3	**Lyse**	Add 6 mL Lysis Buffer (L7). Mix gently by inverting the capped tube until the mixture is homogeneous. Do not vortex. Incubate at room temperature for 5 minutes. **Note**: If using Lysis Indicator, the solution turns blue.
4	**Precipitate**	Add 6 mL Precipitation Buffer (N3). Mix immediately by inverting the tube until the mixture is homogeneous. Do not vortex. **Note**: If using Lysis Indicator, the solution turns completely colorless.
5	**Clarify**	Pour the lysate into a **Lysate Clarification Column** (pre-inserted in a 50-mL conical tube with white cap). Centrifuge at 1,000 × *g* for 4 minutes.
6	**Remove Endotoxins**	Remove and discard the Lysate Clarification Column. 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.
7	**Bind**	Attach the **DNA-binding Column** to a vacuum manifold and load the **clarified lysate** (from step 6) onto the column. Apply vacuum to drain the solution.
8	**Wash**	Add 20 mL (one column volume) of Wash Buffer (W8) to the DNA-binding Column and apply vacuum to drain the solution.
9	**Elute**	Place the DNA-binding Column into an endotoxin-free 50-mL conical tube (with blue cap). Add 15 mL Elution Buffer (E4) to the column and incubate for 1 minute. Centrifuge at 1,000 × *g* for 1 minute. Remove and discard the column.
10	**Precipitate and Wash**	Add 10.5 mL (0.7 volume) of isopropanol to the eluate. Mix well. Centrifuge at >12,000 × *g* for 30 minutes at 4°C. Remove and discard the supernatant. Wash the DNA pellet with 5 mL 70% ethanol. Centrifuge at >12,000 × *g* for 10 minutes at 4°C. Remove the supernatant.
11	**Resuspend**	Air-dry the pellet for 10 minutes, then resuspend the purified plasmid DNA in TE Buffer (TE). Store plasmid DNA in endotoxin-free tubes at –20°C.

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12 February 2017

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