PureLink™ Expi Endotoxin-Free Giga Plasmid Purification Kit

QUICK REFERENCE

invitrogen

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Package Contents

Catalog Numbers	Amount:
A31233	2 preps

Storage Conditions

- Store all components at room temperature.

Required Materials

- Vacuum source capable of –600 to –800 mbar
- Appropriately sized tubes and bottles
- 1000-mL Stericup™ Receiver flask or 45-mm thread sterile glass bottle
- 250-mL Stericup™ Receiver flask or 45-mm thread sterile glass bottle
- Centrifuge and rotor capable of >12,000 x *g* at 4°C

Timing

Bacterial culture: overnight
Purification: 120 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 Giga 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 15 mg of high quality, endotoxin-free ultrapure plasmid DNA from 2.5–5 L of bacterial culture.
- Endotoxin-free (<0.1 EU/µg) DNA with ultra-low levels of RNA, gDNA or protein contamination that is deal 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 RNase A to the Resuspension Buffer (R3) and mix well (see instructions on label). Indicate that RNase A has been added on the bottle label. Store at 4°C.
- 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 2.5 L (high copy number plasmid) or 5 L (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**.

Gigaprep plasmid isolation protocol

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 125 mL Resuspension Buffer (R3) with RNase A to the cell pellet and resuspend the pellet until it is homogeneous.
3	**Lyse**	Add 125 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 125 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 filtration cartridge** attached to a **receiver flask**. Incubate for 2 minutes. Connect a vacuum source and filter the lysate.
6	**Wash**	Add 50 mL Wash Buffer (W8) to the filtration cartridge and gently stir precipitate with a spatula. Apply vacuum. The **clarified lysate contains the plasmid DNA**.
7	**Remove Endotoxin**	Add 30 mL Endotoxin Removal Buffer (ER) to the clarified lysate and mix by inverting the bottle 10 times.
8	**Equilibrate**	Add 200 mL Equilibration Buffer (EQ1) to a **DNA-binding cartridge** attached to a **receiver flask**. Connect a vacuum source and drain the cartridge.
9	**Bind**	Load the **clarified lysate** (from step 7) onto the DNA-binding cartridge. Apply vacuum and drain solution.
10	**Wash**	Add 275 mL Wash Buffer (W8) and apply vacuum. Repeat wash step. Attach **DNA-binding cartridge** to a **new receiver flask**.
11	**Elute**	Add 100 mL Elution Buffer (E4) to the DNA-binding cartridge. Apply vacuum and draw 10–20 mL of solution. Stop the vacuum and incubate for 1 minute. Apply vacuum until all the liquid has passed from the cartridge.
12	**Precipitate and Wash**	Make aliquots of the eluate and add 0.7 volume of isopropanol to each tube. Mix well. Centrifuge at >12,000 × *g* for 30 minutes at 4°C. Remove and discard the supernatant. Wash the DNA pellets in 5 mL 70% ethanol for each tube. Centrifuge at >12,000 × *g* for 10 minutes at 4°C. Remove the supernatant.
13	**Resuspend**	Air-dry the pellets 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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