PureLink® HiPure Expi Megaprep Kit

invitrogen
by Thermo Fisher Scientific

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Package Contents

Catalog Numbers	Amount:
K210008XP	4 preps

Storage Conditions

- Store all components at room temperature.

Required Materials

- Vacuum source equipped with regulator (capable of–600 to–800 mbar)
- Appropriately sized tubes and bottles
- 1000-mL Stericup® Receiver flask
- 250-mL Stericup® Receiver flask
- Centrifuge and rotor capable of >12,000 x *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™ Expression System

Product Description

- The PureLink® HiPure Expi Megaprep Kit provides users with the ability to isolate large quantities of transfection-grade plasmid DNA using an enhanced anion exchange resin. The kit includes filtration columns to provide bacterial filtration without centrifugation in a single unit.
- The PureLink® HiPure Expi Megaprep Kit typically isolates 4 mg of high quality, ultrapure plasmid DNA with inherently low endotoxin levels from 500 mL of bacterial culture.
- High Yield–Isolate over 5 mg of high quality plasmid DNA from a single purification using 1 L of bacterial culture volume.
- Purity–Low endotoxin levels (0.1–1.0 EU/µg), and A260/280 >1.8, making it ideal for mammalian cell transfection.

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.
- 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 500 mL (high copy number plasmid) or 2.5 L (low copy number plasmid) of an overnight culture.
- 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 www.lifetechnologies.com/support.

Megaprep Plasmid Isolation Protocol

Steps		Procedure Details
1	Harvest	1. Sediment cells by centrifugation at 4,000 × *g* for 15 min at 4°C. Discard all medium.
2	Resuspend	2. Add 50 mL Resuspension Buffer (R3) with RNase A to the cell pellet and resuspend the pellet until it is homogeneous.
3	Lyse	3. Add 50 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.
4	Precipitate	4. Add 50 mL Precipitation Buffer (N3). Mix immediately by inverting the tube until the mixture is homogeneous. Do not vortex.
5	Clarify	5. 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	6. 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	Equilibrate	7. Add 100 mL Equilibration Buffer (EQ1) to a **DNA-binding cartridge** attached to a **receiver flask**. Connect a vacuum source and drain the cartridge.
8	Bind	8. Load the **clarified lysate** (from step 6) onto the DNA-binding cartridge. Apply vacuum and drain solution.
9	Wash	9. Add 175 mL Wash Buffer (W8) and apply vacuum. Repeat wash step. Attach **DNA-binding cartridge** to a **new receiver flask**.
10	Elute	10. Add 50 mL Elution Buffer (E4) to the DNA-binding cartridge. Apply soft vacuum (–100 to –200 mbar) and draw 10–20 mL of solution. Stop the vacuum and incubate for 1 minute. Apply vacuum to all the liquid has passed from the cartridge.
11	Precipitate and Wash	11. Add 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 in 20 mL 70% ethanol. Centrifuge at >12,000 × g for 10 minutes at 4°C. Remove the supernatant.
12	Resuspend	12. Air-dry the pellet for 10 minutes, then resuspend the purified plasmid DNA in TE Buffer (TE). Store plasmid DNA at –20°C.

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