High-Q™ Midi-Column Plasmid Purification Kit

Ordering info

TBK0193, 25 reactions

Description

High-Q™ Midi-Column Plasmid Purification Kit is an optimized kit to purified plasmid DNA from bacterial culture using an optimized alkaline lysis buffering system and a purification approach based on silica columns in presence of chaotropic salts. Contaminants are efficiently removed during washing step, obtaining a high-quality purified plasmid DNA useful for all downstream procedures.

Features

- High yield and purity, 70-120 µg, A260/A280= 1.8 - 2.0; A260/A230= 1.9 - 2.1.
- Processing time: less than 30 minutes.

Applications

DNA obtained is suitable for downstream molecular biology applications such as restriction enzyme digestion, cloning, PCR, transformation, *in vitro* transcription, sequencing, etc,

Quality Control

DNA isolation from stationary *E. coli* culture is checked by: integrity (agarose gel electrophoresis), quantity and quality (A260/280= 1.8 ± 0.2; A260/230= 2.0 ± 0.2).

Storage

- Store the kit at 25°C.
- Store RNase-A at -20°C.

Material required (not supplied)

Ethanol (CAS 64-17-5).

Kit Components

Components	TBK0193
High-Q™ Midi-Columns with Collection Tubes	25
Elution Tubes (15 mL)	25
RNase A	8 mg [a]
RNase A Resuspension Buffer	1 mL
BP1 Buffer	60 mL[b]
BP2 Buffer	60 mL
BP3 Buffer	60 mL
Binding Buffer	125 mL
WB2 Buffer	25 mL[c]
Elution Buffer	15 mL

Order Info Kit Components: *High-Q™ Midi-Columns with Collection Tubes (TBM0027) | Elution Tubes (TBM0028) | RNase-A (TBZ0318) | RNase-A Resuspension Buffer (TBB0309) | BP1 Buffer (TBB0523) | BP2 Buffer (TBB0524) | BP3 Buffer (TBB0525) | Binding Buffer (TBB0526) | WB2 Buffer (TBB0512) | Elution Buffer (TBB0510).*

Before its use:

[a] Add 0.8 mL RNase A Resuspension Buffer and mix well.

[b] Add 600 µL RNase-A and mix well.

[c] Add 200 mL absolute ethanol and mix well.

PROTOCOL

1. Transfer the bacterial culture into a 50 mL centrifuge tube (not supplied). The volume of the culture should be between 80 and 100 mL.
2. Centrifuge at 6,000 x g for 5 minutes and discard the supernatant.
3. Repeat steps 1-2 using the same tube. Ensure that the supernatant is completely discarded.
4. Add **2 mL BP1 Buffer** and resuspend the pellet thoroughly.

   ✓ *Check RNase A has been added to BP1 Buffer.*
5. Add **2 mL BP2 Buffer** and mix gently by inverting the tube (6-8 times).
6. Incubate the mixture at room temperature about 2 minutes to lyse the cells. Do not exceed 5 minutes of lysis.
7. Add **2 mL BP3 Buffer** and mix gently by inversion (~10 times).
8. Centrifuge at 10,000 x g for 5 minutes.
9. Carefully transfer the supernatant to a clean tube.
10. Add **5 mL Binding Buffer** and mix by inversion (3-5 times).
11. Incubate at room temperature for 2 minutes.
12. Transfer the mix to a **High-Q™ Midi Column** placed in a Collection Tube.
13. Centrifuge at 3,000 x g, 1 minute. Discard the flow-through.
14. Repeat steps 12-13 with the remaining mixture.
15. Place the **High-Q™ Midi Column** into a Collection Tube and add **4 mL WB2 Buffer**.

    ✓ *Check Absolute Ethanol has been added.*
16. Centrifuge at 3,000 x g, 1 minute. Discard the flow-through.
17. To dry the **High-Q™ Midi Column**, place it in the Collection tube and centrifuge again at 3,000 x g for 1 minute.
18. Place the **High-Q™ Midi Column** into an Elution Tube.
19. Add **300-500 µL pre-warmed Elution Buffer** or **Water (Molecular Biology Grade)** on top of the silica membrane.

    Pre-warm Elution Buffer or Water at 70°C.
20. Incubate at room temperature for 2 minutes.
21. Centrifuge at 3,000 x g for 1 minute.
22. Determine DNA quality on agarose electrophoresis gel and quantity by spectrophotometry.
23. Store DNA at -20°C.