High-Q™ Spin-Column Plasmid DNA Purification Kit

Ordering info

TBK0185, 3 reactions (sample)

TBK0186, 50 reactions

TBK0187, 200 reactions

Description

High-Q™-Spin-Column Plasmid DNA 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, 2-38 µg, A260/A280 ~1.8.
- Safe, no phenol extraction.
- Fast, easy and cost-effective protocol.

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; Abs260/230= 2 ± 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	TBK0186	TBK0187
High-Q™ Spin Columns	50	200
Collection Tubes	50	200
RNase A	2 mg [a]	5 mg [b]
RNase A Resuspension Buffer	1 mL	1 mL
BP1 Buffer	15 mL[c]	45 mL[d]
BP2 Buffer	15 mL	45 mL
BP3 Buffer	15 mL	45 mL
Binding Buffer	30 mL	110 mL
WB2 Buffer	8 mL[e]	35 mL[f]
Elution Buffer	15 mL	15 mL

Order Info Kit Components: *Spin Columns (TBM0016) | Collection Tubes (TBM0020) | 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).*

¡WB2 Buffer for samples are ready to use!

Before its use:

[a] Add 0.2 mL RNase A Resuspension Buffer and mix well.

[b] Add 0.5 mL RNase A Resuspension Buffer and mix well.

[c] Add 150 µL RNase-A and mix well.

[d] Add 450 µL RNase-A and mix well.

[e] Add 32 mL absolute ethanol and mix well.

[f] Add 140 mL absolute ethanol and mix well.

PROTOCOL

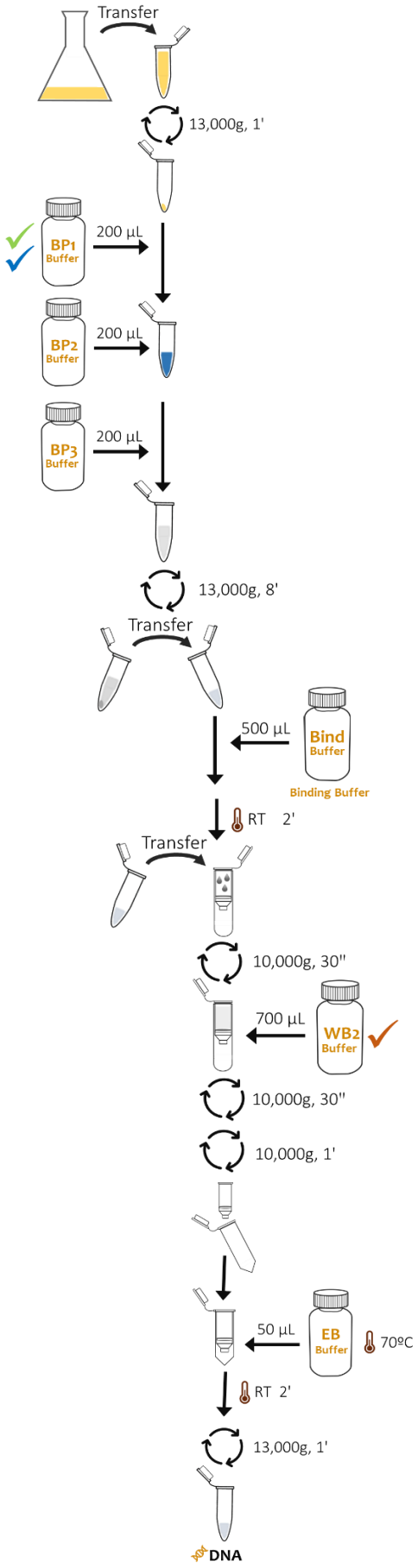
1. Transfer 1.5 mL bacterial culture to a 1.5 mL tube.
2. Centrifuge at 13,000 g for 2 minutes and discard supernatant.
3. Repeat steps 1-2 using the same tube at least once. Ensure to discard the supernatant completely.
4. Add **200 µL BP1 Buffer** and resuspend the pellet.

   ✓ *Check RNase A has been added to BP1 Buffer.*
5. Add **200 µL BP2 Buffer** and mix gently by inverting the tube (6-8 times). Mixing until homogeneous blue color suspension is obtained.
6. Incubate the mixture at room temperature about 2 minutes to lyse the cells. Do not exceed 5 minutes of lysis.
7. Add **200 µL BP3 Buffer** and mix gently by inversion (~10 times).
8. Centrifuge at 13,000 g for 5 minutes.
9. Transfer the supernatant to a clean 1.5 mL tube.
10. Add **500 µL Binding Buffer** and mix by inversion (3-5 times).
11. Incubate at room temperature for 2 minutes.
12. Transfer the mix to a Spin Column placed into a Collection Tube.
13. Centrifuge at 10,000 g, 30 seconds. Discard the flow-through.
14. Repeat steps 12-13 with the remaining mixture.
15. Place the Spin Column into the Collection Tube and add **700 µL WB2 Buffer**.

    ✓ *Check Absolute Ethanol has been added.*
16. Centrifuge at 10,000 g, 30 seconds. Discard the flow-through.
17. To dry Spin Column, place the Spin Column into the Collection Tube and centrifuge again at 10,000 g for 1 minute.
18. Place the Spin Column into a clean 1.5 mL Tube.
19. Add **50-100 µL prewarmed Elution Buffer** or **Water (Molecular Biology Grade)** on top of the silica membrane.

    Prewarm Elution Buffer or Water at 70°C.
20. Incubate at room temperature, 2 minutes.
21. Centrifuge at 10,000 g for 1 minute.
22. Check DNA quality on agarose electrophoresis gel and quantity by spectrophotometry.
23. Store at -20°C.

FLOWCHART PROCEDURE



✓ *RNase A has been added.*

✓ *Ethanol has been added.*