High-Q™-Spin-Column Fungal Genomic DNA Purification Kit

Ordering info

TBK0254, 3 reactions (sample)

TBK0255, 50 reactions

TBK0256, 200 reactions

TBK0257, 250 reactions

Description

High-Q™-Spin-Column Fungal Genomic DNA Purification Kit is an easy silica-membrane-based system for DNA purification from a wide variety of fungal species. An optimized lysis buffer guarantees a good yield while the use of High-Q™ Spin Columns allow a good quality DNA, suitable for downstream applications.

Features

- Easy and Fast protocol.
- Typical yields are **2- 50 µg of genomic DNA**.
- **No organic extraction**, DNA is isolated without phenol, chloroform or ethanol precipitation.
- High DNA purity, the isolated **DNA is ready to use for sensitive downstream molecular biology applications**.
- Isolation of DNA from a **wide range of samples**.

Applications

- Purification of DNA from fungal samples.
- DNA obtained is suitable for downstream molecular biology applications such as PCR, enzymatic digestion for cloning or Southern, genotyping, etc.

Quality Control

DNA purified is checked by: integrity (agarose gel electrophoresis), quantity and quality (A260/280= 1.8 ± 0.2).

Kit Components

Components	TBK0255	TBK0256	TBK0257
High-Q™ Spin Column with Collection Tubes	50	200	250
BFL1 Buffer	25 mL	85 mL	110 mL
BFL1 Additive	3 mL	10 mL	12 mL
BFL2 Buffer	1.5 mL[a]	5 mL[b]	6 mL[c]
BFL3 Buffer	7 mL[d]	28 mL[e]	35 mL[f]
BFL4 Buffer	12 mL[g]	45 mL[h]	2 x 30 mL[i]
Elution Buffer	15 mL	25 mL	25 mL
Proteinase K	1.1 mL*	3 x 30 mg**	4 x 30 mg**
Proteinase K Buffer	-	3 x 1.5 mL	4 x 1.5 mL

Order Info Kit Components: *High-Q™ Spin Columns (TBM0010) | BFL1 Buffer (TBB0547) | BFL1 Additive (TBB0548) | BFL2 Buffer (TBB0549) | BFL3 Buffer (TBB0550) | BFL4 Buffer (TBB0551) | Elution Buffer (TBB0510) | Proteinase K 20 mg/ mL (TBZ0308)* | Proteinase K 30 mg (TBZ0305)** | Proteinase K Buffer (TBB0546).*

¡Components for samples are ready to use!

Before its use:

[a] Add 13.5 mL isopropanol and mix well.

[b] Add 45 mL isopropanol and mix well.

[c] Add 54 mL isopropanol and mix well.

[d] Add 21 mL absolute ethanol and mix well.

[e] Add 84 mL absolute ethanol and mix well.

[f] Add 105 mL absolute ethanol and mix well.

[g] Add 48 mL absolute ethanol and mix well.

[h] Add 180 mL absolute ethanol and mix well.

[i] Add 120 mL absolute ethanol to each bottle. Mix well.

Storage

Store the kit at 25°C and Proteinase K at -20°C.

Material required (not supplied)

- 1.5 mL Tubes.
- Ethanol (CAS 64-17-5).
- Isopropanol (CAS 67-63-0).

PROTOCOL

1. Grind up to 100 mg of fungal mycelia in liquid nitrogen using a mortar and a pestle. With a freeze spatula, collect the powder into a 1.5mL tube. Commercially available equipment for homogenization can also be used.
2. Add **400 µL BFL1 Buffer** and **40 µL BFL1 Additive**. Vortex briefly.
3. Add **20 µL Proteinase K (20 mg/ mL)**. Mix well. Incubate at 65°C, 30 minutes. Mix 2-3 times by inversion during incubation.
4. Centrifuge at 13,000 g for 5 minutes. Transfer the supernatant to a fresh tube.
5. *[Optional] Add **10 µL RNase-A (20 µg/ µL)** and incubate for 5 minutes at room temperature.*
6. Add **200 µL BFL2 Buffer** and mix by inversion.
   Check isopropanol has been added to BFL2 Buffer (✔).
7. Transfer **up 700 µL mixture** to a High-Q™ Spin Column placed into a Collection Tube. Incubate 2 minutes at room temperature.
8. Centrifuge at 10,000 g for 1 minute. Remove the flow-through and place back the High-Q™ Spin Column into a Collection Tube. Repeat steps 7 and 8 with the remaining mixture.
9. Add **500 µL BFL3 Buffer**.
   Check absolute ethanol has been added to BFL3 Buffer (✔).
10. Centrifuge at 10,000 g for 1 minute. Discard the flow-through.
11. Place back the High-Q™ Spin Column into the Collection Tube and add **500 µL BFL4 Buffer**. Repeat this step one more time.
    Check absolute ethanol has been added to BFL4 Buffer (✔).
12. To dry High-Q™ Spin Column and eliminate residual ethanol, centrifuge again at 10,000 g for 1 minute.
13. Place the High-Q™ Spin Column into a clean 1.5 mL Tube.
14. Add **50-100 µL prewarmed Elution Buffer** or **Water** (Molecular Biology Grade).
    Prewarm Elution Buffer or Water at 70°C.
15. Incubate at room temperature, 2 minutes.
16. Centrifuge at 10,000 g for 1 minute to elute purified DNA.
17. Check DNA quantity by spectrophotometry and quality on agarose electrophoresis gel.
18. Store DNA at -20°C.