High-Q™-Spin-Column Keratinous Tissue DNA Purification Kit

(Hair, Nails & Feathers)

Ordering info

TBK0171. 3 reactions (sample)

TBK0172. 50 reactions

TBK0173. 200 reactions

TBK0174. 500 reactions

Description

High-Q™-Spin-Column Keratinous Tissue DNA Purification Kit is an easy silica-membrane-based system for DNA purification from keratinous samples including hair, nails & feathers. Key to the extraction of DNA from keratinous tissues is to break down the keratin in order to liberate the DNA. This is achieved by using an optimized lysis buffers that contain large amounts of detergents and reducing agents such as DTT and proteinase K. The DNA binds to the spin column, while cellular debris and other proteins are effectively washed away by DNA Wash Buffer. Pure DNA is then eluted using sterile deionized water or elution buffer.

Features

- No organic extraction, no ethanol precipitation.
- High DNA purity, the isolated DNA is ready to use for downstream molecular biology applications.
- Easy and Fast protocol.

Applications

Extraction of genomic DNA from Hair, Nails & Feathers.

Quality Control

DNA purified is checked by: integrity (agarose gel electrophoresis), quantity and quality (A260/280= 1.9 ± 0.2).

Kit Components

Components	TBK0172	TBK0173	TBK0174
High-Q™ Spin Column with Collection Tubes	50	200	500
Tissue-1 Buffer	30 mL	110 mL	3 x 100 mL
Tissue-2 Buffer	12 mL[a]	40 mL[b]	2 x 44 mL[c]
WB2 Buffer	12 mL[d]	45 mL[e]	2 x 50 mL[f]
Elution Buffer	15 mL	25 mL	2 x 25 mL
DTT Solution 1M	1.5 mL	4 x 1.5 mL	3 g [g]
Proteinase K	2 x 30 mg [h]	6 x 30 mg [h]	2 x 300 mg [i]
Proteinase K Resuspension Buffer	2 x 1.5 mL	6 x 1.5 mL	30 mL

Order Info Kit Components: *High-Q™ Spin Column with Collection Tubes (TBM0010) | Tissue-1 Buffer (TBB0579) | Tissue-2 Buffer (TBB0580) | WB2 Buffer (TBB0512) | Elution Buffer (TBB0510) | DTT Solution 1M (TBR0150) | Proteinase K (TBZ0303) | Proteinase K Resuspension Buffer (TBB0546).*

¡Components for samples are ready to use!

Before its use:

[a] Add 18 mL isopropanol and mix well.

[b] Add 60 mL isopropanol and mix well.

[c] Add 66 mL Isopropanol and mix well.

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

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

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

[g] DTT Solution 1M: Add 20 mL Nuclease Free Water

[h] Add 1.5 mL Proteinase K Resuspension Buffer and mix well.

[i] Add 15 mL Proteinase K Resuspension Buffer and mix well.

Storage

Store the kit at 25°C.

Store Proteinase K Solution and 1M DTT Solution at -20°C.

Material required (not supplied)

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

PROTOCOL

1. Add **500 µL Tissue-1 Buffer**, **50 µL Proteinase K** (20 mg/mL) and **50 µL 1M DTT Solution** to a 1.5mL microcentrifuge tube.

   DTT oxidizes quickly in aqueous solutions and should also be added just before use. Store the DTT stock solution (1M) at –20°C.

- Cut the sample (up to 50 mg) into small pieces, and transfer it to the microcentrifuge tube from step 1. Close the lid and mix thoroughly by vortexing for 10 s.
- Cut a 2–5 cm piece from the feather (up to 50 mg), and transfer it to the microcentrifuge tube from step 1. Close the lid and mix thoroughly by vortexing for 10 s.

  Select the primary feathers as sample.
- Cut off a 0.5–1 cm piece from the base of the hair, and transfer it to the microcentrifuge tube from step 1. Use 10-15 pieces. Close the lid and mix thoroughly by vortexing for 10 s.

2. Incubate at 56°C, 60 minutes or until the sample is completely lysed. Lysis time varies depending on the sample processed. Mix 2-3 times by inversion during incubation.

   Overnight lysis is possible, although 16 h should not be exceeded.

3. Centrifuge at 13,000 g for 5 minutes. Transfer the supernatant to a new microcentrifuge tube (Not provided).

4. *[Optional] Add* ***20 µL RNase-A*** *20 µg/ µL (not provided) and incubate for 5 minutes at room temperature.*

5. Add **400 µL Tissue-2 Buffer** and mix by inversion.

   Check isopropanol has been added to Tissue-2 Buffer (✔).

6. Transfer up **700 µL mixture** to a High-Q™ Spin Column placed into a Collection Tube.

7. 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.

8. Add **500 µL WB2 Buffer**.

   Check absolute ethanol has been added to WB2 Buffer (✔).

9. Centrifuge at 10,000 g for 30 seconds. Discard the flow-through and place back the High-Q™ Spin Column into a Collection Tube. Repeat steps 9 and 10 one more time.

10. To dry Spin Column and eliminate residual ethanol, centrifuge again at 10,000 g for 1 minute.

11. Place the High-Q™ Spin Column into a clean 1.5 mL Tube. Add **50-100 µL prewarmed Elution Buffer** or **Water (Molecular Biology Grade)**.

    Prewarm Elution Buffer or Water at 70°C.

12. Incubate at room temperature, 2 minutes.

13. Centrifuge at 10,000 g for 1 minute to elute purified DNA.

14. Store DNA at -20°C.