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

Ordering info

TBK0420-S, 3 reactions (sample)

TBK0420, 20 reactions

TBK0421, 50 reactions

TBK0422, 100 reactions

Description

High-Q™-Spin-Column Sperm Genomic DNA Purification Kit is a reliable, silica membrane-based system designed for the efficient purification of high-quality genomic DNA from sperm samples. The kit utilizes the reversible nucleic acid-binding properties of the High-Q™ silica matrix, enabling streamlined binding, washing, and elution steps.

Following lysis, genomic DNA selectively binds to the advanced silica gel membrane, while proteins and other contaminants are efficiently removed through wash steps. Two optimized wash buffers ensure the removal of impurities, leaving highly pure DNA ready for downstream applications after elution in the provided buffer.

Features

- Safety, no phenol extraction, no ethanol precipitation required.
- High yield and purity.
- Isolated DNA is ready to use for downstream molecular biology applications.
- Easy and fast protocol.

Applications

- Genomic DNA purification from horse and bull sperm has been validated.
- DNA obtained is suitable for downstream molecular biology applications such as PCR, qPCR, Southern, enzymatic DNA restriction, etc.

Kit Components

Components	TBK0420	TBK0421	TBK0422
High-Q™ DNA Spin Column with Collection Tubes	20	50	100
PBS 1x pH 7.4	60 mL	1 pouch[a]	1 pouch[a]
Sperm Lysis Buffer	10 mL	25 mL	50 mL
Sperm Washing Buffer	6 mL[b]	12 mL[c]	24 mL[d]
WB2 Buffer	5 mL[e]	12 mL[f]	24 mL[g]
Elution Buffer	2 mL	10 mL	10 mL
DTT 1M	1 mL	1.5 mL	2x 1.5 mL
Proteinase K	20 mg [h]	30 mg [i]	2x 30 mg [i]
Proteinase K Resuspension Buffer	1.5 mL	1.5 mL	2x 1.5 mL

Order Info Kit Components: *High-Q™ DNA Spin Columns (TBM0010) |PBS 1x, pH 7.4 (TBB0600) |Sperm Lysis Buffer (TBK0420-1) | Sperm Washing Buffer (TBK0420-2) | WB2 Buffer (TBB0512) | Elution Buffer (TBB0510) | DTT (TBR0150) | Proteinase K (TBZ0303) | Proteinase K Resuspension Buffer (TBB0546).*

¡Components for samples are ready to use!

Before its use:

[a] Add 1 L distilled water and mix well.

[b] Add 9 mL absolute ethanol and mix well.

[c] Add 18 mL absolute ethanol and mix well.

[d] Add 36 mL absolute ethanol and mix well.

[e] Add 20 mL absolute ethanol and mix well.

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

[g] Add 96 mL absolute ethanol and mix well.

[h] Add 1 mL Proteinase K Resuspension Buffer and mix well.

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

Storage

Store the kit at 25°C.

Store proteinase K solution and DTT at -20°C.

Material required (not supplied)

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

PROTOCOL

1. In a 1.5 mL microcentrifuge tube, add **200 µL Sperm.**
2. Centrifuge at 5,000 g for 2 min and resuspend the pellet in **1 mL PBS 1x pH 7.4**. Discard the supernatant.
3. Wash the pellet 2 times: Resuspend the pellet in **1 mL PBS 1x pH 7.4** and centrifuge at 5,000 g for 2 min. Discard the supernatant.
4. Resupend the pellet in **450 µL Sperm Lysis Buffer**.
5. *[Optional]* Add **20 µL RNasa (10 m g/mL)**.
   This step is necessary only if RNA-free genomic DNA is required.
6. Add **25 µL DTT 1M** and **20 µL Proteinase K Solution (20 mg/mL).**
7. Incubate at 60°C for 1.5-2 hours.
8. Add **300 µL Isopropanol** and mix well by inversion.
9. Transfer up **700 µL mixture** to a High-Q™ DNA Spin Column placed into a Collection Tube.
10. Centrifuge at 10,000 g for 1 minute. Remove the flow-through and place back the High-Q™ DNA Spin Column into a Collection Tube. If necessary, repeat steps 9 and 10 with the remaining mixture.
11. Add **500 µL Sperm Washing Buffer** (✓) and centrifuge at 10,000 g for 1 minute. Discard the flow-through and place the High-Q™ DNA Spin Column back into the Collection Tube.
    ✓ *Check Ethanol has been added.*
12. Add **500 µL WB2 Buffer** (✓).
    ✓ *Check Ethanol has been added.*
13. Centrifuge at 10,000 g for 1 minute. Discard flow-through. Place High-Q™ DNA Spin Column back in the Collection Tube and repeat once steps 12-13.
14. To dry silica matrix, centrifuge at 10,000g for 1 minute.
15. Place High-Q™ DNA spin column into a clean 1.5-mL microcentrifuge tube.
16. Carefully and without touching the matrix, add in the center of High-Q™ DNA Spin Column, **50 µL Elution Buffer** prewarmed at 65°C.
17. Incubate at room temperature for 2 minutes.
18. Centrifuge for 1 minute at 13,000 g. DNA isolated is in the eluate. Discard High-Q™ DNA Spin Column.
19. Store purified DNA at -20°C.