High-Q™-Spin-Columns Blood & Cell Culture Genomic DNA Purification Kit

Ordering info

TBK0121, 3 reactions (sample)

TBK0122, 50 reactions

TBK0123, 200 reactions

Description

High-Q™-Spin-Columns Blood & Cell Culture Genomic DNA Purification Kit is a silica-membrane-based DNA purification kit. to obtain genomic DNA with high quality and purity. Suitable for blood, plasma, serum and other body fluids as well as for cell culture samples.

Features

- High yield and purity, 2-15 µg, A_{260}/A_{280} = 1.8 ± 0.2; A_{260}/A_{230} = 2.0 ± 0.2.
- Suitable for wide variety of samples like fresh or frozen anticoagulated mammalian blood (treated with EDTA, Citrate or Heparine), aged blood, plasma, serum.
- Complete Blood Sample from 25 µL to 200 µL.

Applications

DNA obtained is suitable for downstream molecular biology applications such as real-time PCR, endpoint PCR, multiplex PCR and enzymatic digestion for cloning or Southern, genotyping, etc.

Quality Control

DNA isolation from 200 µL whole human blood is checked by: integrity (agarose gel electrophoresis), quantity and quality ($A_{260/280}$= 1.8 ± 0.2).

Material required (not supplied)

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

Kit Components

Components	TBK0122	TBK0123
High-Q™ Spin Column with Collection Tubes	50	200
Blood Lysis Buffer	20 mL	65 mL
PBS 1x pH 7.4	15 mL	60 mL
Blood Binding Buffer	8 mL[a]	26 mL[b]
Blood Washing Buffer	12 mL[c]	44 mL[d]
WB2 Buffer	16 mL[e]	2 x 30 mL[f]
Elution Buffer	15 mL	25 mL
Proteinase K	30 mg [g]	4 x 30 mg [g]
Proteinase K Resuspension Buffer	1.5 mL	4 x 1.5 mL

Order Info Kit Components: *High-Q™ Spin Column with Collection Tubes (TBM0010) | Blood Lysis Buffer (TBB0498) | PBS (TBB0360) | Proteinase K (TBZ0305) | Proteinase K Resuspension Buffer (TBB0546) | Blood Binding Buffer (TBB0499) | Blood Washing Buffer (TBB0500) | WB2 Buffer (TBB0512) | Elution Buffer (TBB0510).*

Before its use:

[a] Add 12 mL isopropanol and mix well.

[b] Add 39 mL isopropanol and mix well.

[c] Add 18 mL isopropanol and mix well.

[d] Add 66 mL isopropanol and mix well.

[e] Add 64 mL absolute ethanol and mix well

[f] Add 120 mL absolute ethanol and mix well.

[g] Add 1.5 mL Proteinase K Resuspension Buffer and mix well. Store at -20°C.

Storage

Store the kit at 25°C.

Store Proteinase K at -20°C.

PROTOCOL FOR GENOMIC DNA PURIFICATION

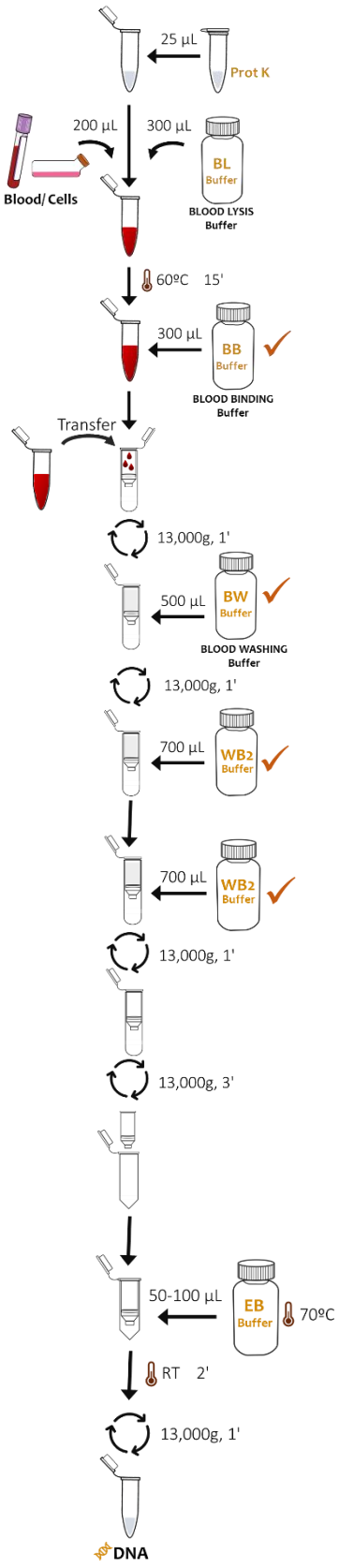
I. FROM BLOOD

1. Transfer **25 µL Proteinase K** to a 1.5 mL tube *(not provided)*.
2. Add to up **200 µL Sample.**
3. *Optional: add **10 µL RNase (20 mg/mL)** (not provided).*
4. Add **300 µL Blood Lysis Buffer** and mix by vortex until homogenous solution is observed.
5. Incubate at 60°C, 15 minutes and shake occasionally.
6. Add **300 µL Blood Binding Buffer** and mix by vortex vigorously.

   ✔*Check isopropanol has been added to Blood Binding Buffer.*
7. Place a High-Q™ Spin Column into a Collection Tube and transfer lysate from Step 6 to the spin column reservoir using a pipette.
8. Centrifugate at 13,000 g for 1 minute and discard the flow-through.
9. Add **500 µL Blood Washing Buffer**.

   ✔*Check isopropanol has been added to Blood Washing Buffer.*
10. Centrifugate at 13,000 g for 1 minute and discard the flow-through.
11. Add **700 µL WB2 Buffer.**

    ✔*Check absolute ethanol has been added to WB2 Buffer.*
12. Centrifugate at 13,000 g for 1 minute and discard the flow-through. Repeat steps 11 and 12 one more time.
13. Centrifugate again at 13,000 g for 2 minutes to remove residual ethanol.
14. Place the reservoir into a clean 1.5 mL tube.
15. Add **50-100 µL EB Buffer** or **Water, Molecular Biology Grade** (pre-heated at 70°C) on top of the silica membrane.
16. Incubate at room temperature for 2 minutes.
17. Centrifugate at 13,000 g for 1 minute to collect DNA in eluate.



II. FROM CULTURED CELLS

1. Transfer **5x10^6 cells** to a 1.5 mL tube.
2. Centrifuge at 3,000 g for 5 minutes and discard supernatant.
3. Add **200 µL PBS 1x pH 7.4** to the cell pellet and resuspend the pellet.
4. In a tube containing **25 µL Proteinase K**, add **200 µL resuspended cells** from previous step. Continue at **Step 3** of the previous procedure.

High-Q™-Spin-Columns Blood/ Cell Culture Genomic DNA Purification Kit

Referencias

TBK0121, 3 reacciones (muestra)

TBK0122, 50 reacciones

TBK0123, 200 reacciones

Descripción

High-Q™-Spin-Columns Blood & Cell Culture Genomic DNA Purification Kit es un kit de purificación de ADN basado en el uso de una matriz de sílica, diseñado para obtener ADN genómico de alta calidad y pureza. Adecuado para muestras de sangre, plasma, suero y otros fluidos corporales, así como para muestras de cultivos celulares.

Características

- Alto rendimiento y pureza, 2-15 µg; A260/A280 = 1,8 ± 0,2; A260/A230 = 2,0 ± 0,2.
- Disponible para una amplia variedad de muestras de sangre de mamíferos no coagulada (tratada con EDTA, citrato o heparina), fresca o congelada, plasma, suero, etc.
- Uso de sangre completa (25-200 µL) como muestra.

Aplicaciones

El ADN obtenido puro puede ser utilizado para aplicaciones en biología molecular como PCR en tiempo real, PCR a tiempo final, PCR multiplex, digestiones enzimáticas para clonaciones, genotipado.

Control de Calidad

El aislamiento de ADN de sangre humana completa se verifica mediante: integridad (electroforesis en gel de agarosa), cantidad y calidad (A260/280 = 1,8 ± 0,2).

Material requerido (no suministrado)

- Etanol (CAS 64-17-5)
- Isopropanol (CAS 67-63-0)

Componentes

Componentes	TBK0122	TBK0123
High-Q™ Spin Column with Collection Tubes	50	200
Blood Lysis Buffer	20 mL	60 mL
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Antes de usar:

[a] Añadir 12 mL isopropanol y mezclar bien.

[b] Añadir 39 mL isopropanol y mezclar bien.

[c] Añadir 18 mL isopropanol y mezclar bien.

[d] Añadir 66 mL isopropanol y mezclar bien.

[e] Añadir 64 mL etanol absoluto y mezclar bien.

[f] Añadir 120 mL etanol absoluto y mezclar bien.

[g] Añadir 1,5 mL Proteinasa K Resuspension Buffer y mezclar bien. Conservar a -20°C.

Almacenamiento

Conservar el kit a 25°C.

Conservar Proteinase K a -20°C.

PROTOCOLO

I. A PARTIR DE SANGRE

1. Transferir **25 µL Proteinase K** a un tubo de 1,5 mL.
2. Añadir hasta **200 µL Muestra.**
3. *Opcional: añadir **10 µL RNase (20 mg/mL)** (no suministrado).*
4. Añadir **300 µL Blood Lysis Buffer** y mezclar con vortex hasta observar una solución homogénea.
5. Incubar a 60°C, 15 minutos y agitar ocasionalmente.
6. Añadir **300 µL Blood Binding Buffer** y mezclar vigorosamente con vortex.

   ✔*Comprobar que ha sido añadido el isopropanol al Blood Binding Buffer.*
7. Colocar un High-Q™ Spin Column dentro de un Collection Tube y transferir el lisado obtenido en el paso 6 al reservorio de la columna usando una pipeta.
8. Centrifugar a 13.000 g durante 1 minuto y eliminar el eluato.
9. Añadir **500 µL Blood Washing Buffer**.

   ✔*Comprobar que ha sido añadido el isopropanol al Blood Washing Buffer.*
10. Centrifugar a 13.000 g durante 1 minuto y eliminar el eluato.
11. Añadir **700 µL WB2 Buffer.**

    ✔*Comprobar que ha sido añadido el etanol absoluto al WB2 Buffer.*
12. Centrifugar a 13.000 g durante 1 minuto y eliminar el eluato. Repetir los pasos 11 y 12 una vez más.
13. Centrifugar a 13.000 g durante 2 minutos para eliminar el etanol residual.
14. Colocar el reservorio en un tubo limpio de 1,5 mL.
15. Añadir **50-100 µL EB Buffer** o **Water, Molecular Biology Grade** (pre-calentada a 70°C) sobre la superficie de la matriz de sílica.
16. Incubar a temperatura ambiente durante 2 minutos.
17. Centrifugar a 13.000 g durante 1 minuto y recoger el eluato.

II. A PARTIR DE CÉLULAS CULTIVADAS

5. Transferir **5x10^6 células** a un tubo de 1,5 mL.
6. Centrifugar a 3.000 g durante 5 minutos y eliminar el sobrenadante.
7. Añadir **200 µL PBS 1x pH 7,4** al pellet y resuspender.
8. En un tubo con **25 µL Proteinase K**, añadir **200 µL células resuspendidas** del paso previo. Continuar en el paso 3 del protocolo anterior.