Lumiprobe
Life science solutions

LumiSpin® BB, DNA Isolation Spin Kit for Blood and Buccal Swab manual

Contents

EN

LumiSpin® BB, DNA Isolation Spin Kit for Blood and Buccal Swab manual

The Kit allows rapid (about 30 minutes) and highly efficient purification of total DNA (genomic, mitochondrial, and viral) from whole blood, buccal swabs, leukocytes, cultured mammalian cells using spin columns. The isolated DNA is stable and suitable for PCR, restriction enzyme digestion, Southern blotting, preparing samples for Sanger sequencing and NGS, etc.

Typical yield of genomic DNA (30–50 kb in size, OD_{260}/OD_{280} 1.7–1.8):

- whole blood (100 µL): 1–3 µg
- buccal swab: 0.5–2 µg
- cultured mammalian cells (1×10^6): 3–6 µg

Kit components

Kit component	Count		
	11553 10 minipreps	21553 50 minipreps	31553 100 minipreps
12164, Spin column (up to 20 µg), 10 pcs	1	—	—
F4150, Lysis Solution BB, 4 mL	1	—	—
H2450, Wash Solution A (with GuHCl), 6 mL	1	—	—
M4150, Lysis Solution BB, 20 mL	—	1	—
P2450, Wash Solution A (with GuHCl), 30 mL	—	1	—
R4150, Lysis Solution BB, 40 mL	—	—	1
T2450, Wash Solution A (with GuHCl), 60 mL	—	—	1
M2250, Wash Solution B (Concentrate to be diluted 5x with ethanol 96%), 20.0 mL	—	—	1

32850, Proteinase K (lyophilized), 12.0 mg	—	—	1
D3850, Proteinase K Dilution Buffer, 1200 uL	—	—	1
Collection tube for spin column, 2 mL	30	150	300
K2250, Wash Solution B (Concentrate to be diluted 5x with ethanol 96%), 10.0 mL	1	1	—
D1350, Elution Buffer (10 mM Tris-HCl, pH 8.5), 1.5 mL	1	4	8
12850, Proteinase K (lyophilized), 6.0 mg	1	1	—
B3850, Proteinase K Dilution Buffer, 600 uL	1	1	—
22164, Spin column (up to 20 µg), 50 pcs	—	1	2

Store at room temperature.

Shelf life 12 months.

Equipment and reagents supplied by user:

- heating block (alternatively, a water bath can be used);
- microcentrifuge with rotor for 1.5 mL tubes and capable of centrifuging >10,000 RPM (6,700 × g);
- 96 % ethanol;
- 1.5 mL microcentrifuge tubes (2 tubes for DNA purification from 1 biological sample); and
- phosphate-buffered saline (PBS) for DNA purification from cultured mammalian cells.

Before starting

1. Dilute the concentrate of *Wash Solution B* 5x with 96 % ethanol (add 4 volumes of 96 % ethanol to 1 volume of concentrate specified on the bottle). Mark on the label that ethanol has been added.

2. Add 600 µL of *Proteinase K Dilution Buffer* into the vial containing lyophilized *Proteinase K*. Mix thoroughly and centrifuge briefly.

   ! Store Proteinase K solution after preparation at -20 °C.

3. If any precipitate has formed in *Lysis Solution BB* or *Wash Solution A*, incubate the solutions at a temperature not higher than 50 °C until the precipitates have been completely dissolved.

All centrifugation steps are carried out at room temperature,
at 10,000–13,000 RPM (6,700–11,000 × g) (unless specified otherwise).

DNA purification from whole blood

This kit is designed to process 100 µL of whole blood (fresh or frozen whole blood containing anti-coagulants such as heparin, EDTA, citrate). For increased DNA yield, prior separation of leukocytes can be performed (a whole blood sample volume recommended for leukocyte separation is 1–2 mL).

1. Set a heating block to 55 °C, place the vial with *Elution Buffer* in the heating block.
2. Mix blood tubes thoroughly by inversion so that the content becomes homogeneous. Pipet 100 µL of whole blood into a clean 1.5 mL tube.
3. Add 300 µL of *Lysis Solution BB* and 10 µL of *Proteinase K* to the sample, mix well by vortexing.
4. Incubate the tubes at 55 °C for 15 min with occasional vortexing (1–2 times).
5. Place a spin column in a collection tube. Add the lysate to the column. Centrifuge the column for 45 seconds. Discard the collection tube and place the spin column into a clean collection tube.
6. Add 500 µL of *Wash Solution A*, centrifuge the column for 30 seconds. Discard the collection tube and place the spin column into a clean collection tube.
7. Add 500 µL of *Wash Solution B*, centrifuge the column for 3 minutes. Discard the collection tube.
8. Place the spin column into a clean 1.5 mL microcentrifuge tube. Add 50–200 µL of *Elution Buffer* (pre-heated to 55 °C) to the center of the spin column membrane. Incubate at room temperature for 2 minutes. Centrifuge the column for 1 minute. The microcentrifuge tube contains the purified DNA.

DNA purification from buccal swabs

1. Set a heating block to 55 °C, place the vial with *Elution Buffer* in the heating block.
2. Add 300 µL of *Lysis Solution BB* to a clean 1.5 mL microcentrifuge tube.
3. Rinse a buccal swab sample in *Lysis Solution BB* thoroughly. Use a rolling action against the tube sides and squeeze the swab against side to remove as much of the liquid as possible.
4. Add 10 µL of *Proteinase K* to the sample, mix well by vortexing.
5. Incubate the tubes at 55 °C for 15 min with occasional vortexing (1–2 times).
6. Place a spin column in a collection tube. Add the lysate to the column. Centrifuge the column for 45 seconds. Discard the collection tube and place the spin column into a clean collection tube.
7. Add 500 µL of *Wash Solution A*, centrifuge the column for 30 seconds. Discard the collection tube and place the spin column into a clean collection tube.
8. Add 500 µL of *Wash Solution B*, centrifuge the column for 3 minutes. Discard the collection tube.
9. Place the spin column into a clean 1.5 mL microcentrifuge tube. Add 50–200 µL of *Elution Buffer* (pre-heated to 55 °C) to the center of the spin column membrane. Incubate at room temperature for 2 minutes. Centrifuge the column for 1 minute. The microcentrifuge tube contains the purified DNA.

DNA purification from cultured mammalian cells

Use not more than 5×10^6 cells for DNA purification.

Adherent cell culture: remove the growth medium, harvest cells by trypsinization or a method of choice. Centrifuge the cells at 300 × g for 5 minutes. Remove the supernatant. Resuspend the cell pellet in 100 µL of PBS. Transfer the cell suspension to a clean 1.5 mL microcentrifuge tube.

Suspension cell culture: harvest cells by centrifugation at 300 × g for 5 minutes. Remove the supernatant. Resuspend the cell pellet in 100 µL of PBS. Transfer the cell suspension to a clean 1.5 mL microcentrifuge tube.

1. Set a heating block to 55 °C, place the vial with *Elution Buffer* in the heating block.
2. Add 300 µL of *Lysis Solution BB* and 10 µL of *Proteinase K* to the sample, mix well by vortexing.
3. Incubate the tubes at 55 °C for 15 min with occasional vortexing (1–2 times).
4. Place a spin column in a collection tube. Add the lysate to the column. Centrifuge the column for 45 seconds. Discard the collection tube and place the spin column into a clean collection tube.
5. Add 500 µL of *Wash Solution A*, centrifuge the column for 30 seconds. Discard the collection tube and place the spin column into a clean collection tube.
6. Add 500 µL of *Wash Solution B*, centrifuge the column for 3 minutes. Discard the collection tube.
7. Place the spin column into a clean 1.5 mL microcentrifuge tube. Add 50–200 µL of *Elution Buffer* (pre-heated to 55 °C) to the center of the spin column membrane. Incubate at room temperature for 2 minutes. Centrifuge the column for 1 minute. The microcentrifuge tube contains the purified DNA.

Note

For increased DNA concentration, use a lower volume of *Elution Buffer*. Elution with volumes of less than 50 µL is not recommended because this can be insufficient to entirely wet the membrane resulting in low DNA recovery. For increased DNA yield, use a higher volume of *Elution Buffer* (100–200 µL).

Elution Buffer pre-heated to 55 °C facilitates optimal recovery of bound DNA. Elution with *Elution Buffer* equilibrated to room temperature is also acceptable but DNA recovery is significantly lower (up to 30–50 %).

To maximize DNA recovery, two-step elution is recommended.

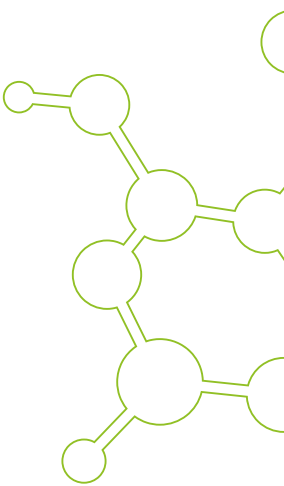
If necessary, DNA can be eluted with deionized water.

When measuring the DNA concentration, dilute the sample only with TE buffer pH 8.5 or *Elution Buffer* supplied with the kit. Otherwise, DNA concentration and purity (OD_{260} and OD_{260}/OD_{280}, respectively) can be estimated incorrectly.

Storage of purified DNA: for long-term storage -20 °C, for short-term storage +4 °C.

Ver. LJX4M
#5555G

22.09.509-QM
Issued by INSPECT



www.lumiprobe.com