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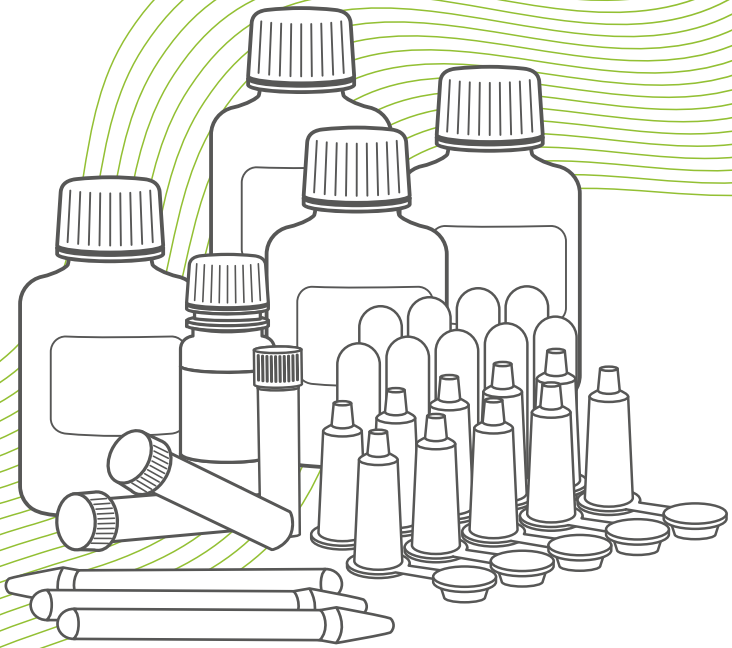

LumiSpin® UNI, DNA Isolation Spin Kit for Any Sample manual

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LumiSpin® UNI, DNA Isolation Spin Kit for Any Sample manual

The kit allows rapid (about 30 minutes) and highly efficient purification of genomic DNA using spin columns from a wide range of biological samples: plant and animal tissues and organs, whole blood, buccal swabs, leukocytes, cultured mammalian cells, and gram-negative bacteria. 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):

- gram-negative bacteria (1×10^8): 5–10 µg
- plant leaves, needles (50 mg): 3–5 µg
- mouse tail: 7–14 µg
- mouse kidney: 10–20 µg
- mouse heart: 7–14 µg
- mouse liver: 10–15 µg
- leukocytes (isolated from 1 mL of whole blood): 4–5 µg
- 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		
	11573 10 minipreps	21573 50 minipreps	31573 100 minipreps
12164, Spin column (up to 20 µg), 10 pcs	1	—	—
H1850, Lysis Solution AS, 6 mL	1	—	—
F5450, Sorption Solution, 4 mL	1	—	—
H2450, Wash Solution A (with GuHCl), 6 mL	1	—	—
K3150, Red Blood Cell Lysis Buffer 10x, 10 mL	1	—	—
11650, RNase A (10 mg/mL), 50 uL	1	—	—
1902-5g, Corundum (50 µm), 5 g	1	—	—
P1850, Lysis Solution AS, 30 mL	—	1	—
M5450, Sorption Solution, 20 mL	—	1	—
P2450, Wash Solution A (with GuHCl), 30 mL	—	1	—
S3150, Red Blood Cell Lysis Buffer 10x, 50 mL	—	1	—
1902-25g, Corundum (50 µm), 25 g	—	1	—
31650, RNase A (10 mg/mL), 150 uL	—	2	—
T1850, Lysis Solution AS, 60 mL	—	—	1
R5450, Sorption Solution, 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
D3850, Proteinase K Dilution Buffer, 1200 uL	—	—	1
32850, Proteinase K (lyophilized), 12.0 mg	—	—	1
1902-50g, Corundum (50 µm), 50 g	—	—	1

U3150, Red Blood Cell Lysis Buffer 10x, 100 mL	—	—	1
51650, RNase A (10 mg/mL), 300 uL	—	—	2
Collection tube for spin column, 2 mL	30	150	300
Pestle for Microcentrifuge Tube (Polypropylene)	10	50	100
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 temperature 20°C.

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 (6700 × g);
- 96 % ethanol;
- 1.5 mL microcentrifuge tubes (2 tubes for DNA purification from 1 biological sample);
- phosphate-buffered saline (PBS) for DNA purification from cultured mammalian cells, leukocytes, gram-negative bacteria;
- tissue grinder for sample homogenization if DNA is purified from plant and animal tissues and organs (if polypropylene pestles for microcentrifuge tubes supplied with the kit are not suitable for user's sample grinding); and
- (optional) tabletop centrifuge with rotor for 15 mL tubes and capable of

centrifuging 300 × g for DNA purification from leukocytes and 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.

   ! After preparation store Proteinase K solution at -20 °C.
3. If any precipitate has formed in *Sorption Solution* 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 (6700–11,000 x g).

DNA purification from plant and animal tissues and organs

Use 20–40 mg of animal tissue or 50–100 mg of plant tissue as a sample for DNA purification.

1. **(A) For sample homogenization with plastic pestle (supplied with the kit):**
   a. Place a piece of tissue into a clean 1.5 mL microcentrifuge tube.
   b. Add to the sample
      - 200 µL of *Lysis Solution AS*,
      - 70–100 mg of *Corundum*.
   c. Grind the sample thoroughly with a tube and pestle.
   d. Add 300 µL of *Lysis Solution AS* to the sample and mix.

1. **(B) For sample homogenization with mortar and pestle (supplied by user):**
   a. Place a piece of tissue into a mortar.
   b. Add to the sample
      - 100 µL of deionized water,
      - 200 µL of *Lysis Solution AS*,
      - 300–500 mg of *Corundum.*
   c. Grind the sample thoroughly with a mortar and pestle.
   d. Add 300 µL of *Lysis Solution AS* to the sample and mix.
   e. Transfer the mixture to a clean 1.5 mL microcentrifuge tube.

1. **(C) For lysis of samples (mouse tails or other samples) without homogenization:**

a. Place a piece of tissue into a clean 1.5 mL microcentrifuge tube.

b. Add to the sample 500 µL of *Lysis Solution AS*.

2. Set a heating block to 55 °C, place the vial with *Elution Buffer* in the heating block.
3. Add to the sample
   - 5 µL of *RNase A*,
   - 10 µL of *Proteinase K*,

   Mix well.
4. **For (A) and (B):** Incubate the tubes at 55 °C for 20 min with occasional vortexing (1–2 times),

   For (C): Incubate the tubes at 55 °C until lysis is complete (1–4 hours with occasional vortexing for small tissue pieces, overnight for mouse tails or larger tissue pieces. Some components such as bones, hair, cartilage that have not been lysed this way can be present in the lysate).
5. Add 350 µL of *Sorption Solution* to the sample. Mix well by vortexing to obtain a homogenous solution.
6. Centrifuge the sample for 10 minutes.
7. Place a spin column in a collection tube. Add the supernatant to the column (to a maximum of 800 µL per single load). Centrifuge the column for 60 seconds. Discard the collection tube and place the spin column into a clean collection tube.
8. 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.
9. Add 500 µL of *Wash Solution B*, centrifuge the column for 3 minutes. Discard the collection tube.
10. Place the spin column into a clean 1.5 mL microcentrifuge tube. Add 50–100 µL of *Elution Buffer* (pre-heated to 55 °C) to the center of the spin column membrane. Incubate at room temperature for 1 minute. 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 500 µL of *Lysis Solution AS* to a 1.5 mL microcentrifuge tube and rinse a buccal swab sample in *Lysis Solution AS* thoroughly. Use a rolling action against the tube sides and squeeze the swab against side to remove as much of the liquid as possible.
3. Add 10 µL of *Proteinase K* to the sample and mix.
4. Incubate the tubes at 55 °C for 10 min with occasional vortexing (1–2 times).
5. Add 300 µL of *Sorption Solution* to the sample. Mix well by vortexing to obtain a homogenous solution.
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 50–100 µL of *Elution Buffer* (pre-heated to 55 °C) to the center of the spin column membrane. Incubate at room temperature for 1 minute. Centrifuge the column for 1 minute. The microcentrifuge tube contains the purified DNA.

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). 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.

1. Set a heating block to 55 °C, place the vial with *Elution Buffer* in the heating block.
2. Add 400 µL of *Lysis Solution AS* to a 1.5 mL microcentrifuge tube containing 100 µL of whole blood.
3. Add 10 µL of *Proteinase K* to the sample and mix.
4. Incubate the tubes at 55 °C for 10 min with occasional vortexing (1–2 times).
5. Add 300 µL of *Sorption Solution* to the sample. Mix well by vortexing to obtain a homogenous solution.
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 50–100 µL of *Elution Buffer* (pre-heated to 55 °C) to the center of the spin column membrane. Incubate at room temperature for 1 minute. Centrifuge the column for 1 minute. The microcentrifuge tube contains the purified DNA.

DNA purification from leukocytes

Prior separation of leukocytes provides an increased DNA yield compared to the DNA purification from whole blood. A whole blood sample volume recommended for leukocyte separation is 1 mL.

1. Prepare 9 mL of 1x *Red Blood Cell Lysis Buffer* for separation of leukocytes from 1 mL of whole blood: mix 900 µL of 10x *Red Blood Cell Lysis Buffer* and 8.1 mL of deionized water in a 15 mL tube.
2. Set a heating block to 55 °C, place the vial with *Elution Buffer* in the heating block.
3. Mix 1 mL of whole blood and 9 mL of 1x *Red Blood Cell Lysis Buffer* in a 15 mL tube. Mix well and incubate the tube for 5 minutes at room temperature.
4. Centrifuge the sample at 300 x g for 5 minutes. Remove the supernatant. The pellet contains the separated leukocytes.
5. Resuspend the cell pellet in 100 µL of PBS. Transfer the cell suspension to a clean 1.5 mL microcentrifuge tube.
6. Add to the sample
   - 400 µL of *Lysis Solution AS*,
   - 5 µL of *RNase A*,
   - 10 µL of *Proteinase K*.

   Mix well.
7. Incubate the tubes at 55 °C for 10 min with occasional vortexing (1–2 times).
8. Add 300 µL of *Sorption Solution* to the sample. Mix well by vortexing to obtain a homogenous solution.
9. 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.
10. 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.

11. Add 500 µL of *Wash Solution B*, centrifuge the column for 3 minutes. Discard the collection tube.
12. Place the spin column into a clean 1.5 mL microcentrifuge tube. Add 50–100 µL of *Elution Buffer* (pre-heated to 55 °C) to the center of the spin column membrane. Incubate at room temperature for 1 minute. Centrifuge the column for 1 minute. The microcentrifuge tube contains the purified DNA.

DNA purification from cultured mammalian cells and gram-negative bacteria

Use not more than 5×10^6 of cultured mammalian cells and 10^9 of gram-negative bacteria cells for DNA purification.

Adherent mammalian cell culture: remove the growth medium, harvest cells by trypsinization or a method of choice. Centrifuge the cells at 300 x 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 mammalian cell culture: harvest cells by centrifugation at 300 x 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.

Bacterial culture: Harvest bacteria grown on solid or in liquid culture medium by centrifugation at 3000–5000 x 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 to the sample
    - 400 µL of *Lysis Solution AS*,

- 5 µL of *RNase A*,
- 10 µL of *Proteinase K*.

Mix well.

3. Incubate the tubes at 55 °C for 10 min with occasional vortexing (1–2 times).
4. Add 300 µL of *Sorption Solution* to the sample. Mix well by vortexing to obtain a homogenous solution.
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 50–100 µL of *Elution Buffer* (pre-heated to 55 °C) to the center of the spin column membrane. Incubate at room temperature for 1 minute. 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 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.

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