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ONESpin™ Universal DNA Extraction Kit (ONE-ULDNA-50)

Intended Use:

The **ONESpin™ Universal DNA Extraction Kit** is intended for the isolation of high-quality genomic DNA from a wide range of human clinical samples, including blood, saliva, urine, vaginal swab, tissue, and faecal specimens. The purified DNA is suitable for downstream molecular applications such as PCR, qPCR, and next-generation sequencing (NGS). This kit is for research use only.

Principle:

The ONESpin™ Universal DNA Extraction Kit utilizes silica membrane spin column technology for the isolation of high-quality genomic DNA from diverse human clinical specimens.

Samples are lysed using a proprietary chaotropic lysis buffer to disrupt cell membranes and release genomic DNA. Under optimized binding conditions, DNA selectively binds to the silica membrane of the spin column, while proteins, polysaccharides, inhibitors, and other contaminants are removed during centrifugation.

The bound DNA is washed with ethanol-containing wash buffers to remove residual impurities, and finally eluted in a low-salt buffer suitable for downstream applications.

Kit Contents:

Components	Quantity (50 Reactions)
CL Buffer	40 mL
BB Buffer	30 mL
Spin Columns	50
BW Buffer (Concentrate)	15 mL
BE Buffer	4 mL

Material's/Equipment's required but not Supplied:

- Ethanol (96–100%), Molecular Grade
- Micropipettes and sterile tips
- 1.5 mL microcentrifuge tubes
- Heating block or Water bath
- Vortex mixer
- Microcentrifuge
- Proteinase K

Storage Conditions:

All components of the **ONESpin™ Universal DNA Extraction Kit** should be stored at room temperature (~25°C). The kit is stable until the expiration date printed on the product label when stored under recommended conditions.

Preparation of Buffers:

Preparation of BW Buffer

BW Buffer is supplied as a **concentrate** and must be diluted with ethanol before use.

Component	Volume for 50 Reactions
BW Buffer (Concentrate)	15 mL
Ethanol (96-100%)	45 mL
Final Volume	60 mL

Mix thoroughly and store at room temperature.

Safety and Precautions:

This kit is for research use only and is not intended for diagnostic or therapeutic applications. All human-derived samples should be considered potentially infectious and handled in accordance with appropriate biosafety guidelines. Wear suitable personal protective equipment**,** including laboratory coat, gloves, and eye protection, when handling samples and reagents. Kit reagents may contain chaotropic salts that are harmful if swallowed, inhaled, or in contact with skin or eyes; avoid direct contact and rinse immediately with water in case of exposure.

A. Whole Blood DNA Extraction Protocol:

1. Add 500 µL CL Buffer to 100 µL whole blood sample and vortex thoroughly.
2. Add Proteinase K to a final concentration of 0.3 mg/mL and vortex well. *(Proteinase K is not supplied with this kit; for example, add 8 µL of 20 mg/mL Proteinase K to 500 µL lysis buffer.)*
3. Incubate at 60 °C for 15 minutes.
4. Invert the tube several times during incubation to ensure proper mixing.
5. Centrifuge at 6,000 rpm for 10 minutes and transfer 400 µL of lysate to a new 1.5 mL tube.
6. Add 400 µL of BB buffer and vortex thoroughly.
7. Transfer the mixture to a spin column placed in a collection tube.
8. Centrifuge at 8,000 rpm for 1 minute. Discard the flow-through and place the column back into the collection tube.
9. If the sample volume exceeds the column capacity, repeat step 7–8 until all the lysate has been processed.
10. Add 500 µL of BW buffer to the spin column and centrifuge at 10,000 rpm for 1 minute. Discard the flow-through.
11. Repeat the wash step once more with BW buffer.
12. Perform a dry spin at 10,000 rpm for 2 minutes to remove residual buffer.
13. Transfer the spin column to a new 1.5 mL microcentrifuge tube.
14. Add 50 µL of BE buffer directly to the center of the membrane.
15. Incubate at room temperature for 5 minutes.
16. Centrifuge at 8,000 rpm for 1 minute to elute DNA. Store the eluted DNA at –20 °C.

B. Saliva DNA Extraction Protocol:

1. Transfer 500 µL of saliva sample into a 1.5 mL microcentrifuge tube and centrifuge at 10,000 rpm for 5 minutes.
2. Carefully discard 400 µL of the supernatant, then add 500 µL CL Buffer and vortex thoroughly.
3. Add Proteinase K to a final concentration of 0.4 mg/mL and vortex well.
   (Proteinase K is not supplied with this kit; for example, add 10 µL of 20 mg/mL Proteinase K to 500 µL lysis buffer.)
4. Incubate at 65 °C for 15 minutes.
5. Invert the tube several times during incubation to ensure proper mixing.
6. Centrifuge at 6,000 rpm for 10 minutes and transfer 500 µL of lysate to a new 1.5 mL tube.
7. Add 500 µL of BB buffer and vortex thoroughly.
8. Transfer the mixture to a spin column placed in a collection tube.
9. Centrifuge at 8,000 rpm for 1 minute. Discard the flow-through and place the column back into the collection tube.
10. If the sample volume exceeds the column capacity, repeat steps 8–9 until all the lysate has been processed.
11. Add 500 µL of BW buffer to the spin column and centrifuge at 10,000 rpm for 1 minute. Discard the flow-through.
12. Repeat the wash step once more with BW buffer.
13. Perform a dry spin at 10,000 rpm for 2 minutes to remove residual buffer.
14. Transfer the spin column to a new 1.5 mL microcentrifuge tube.
15. Add 50 µL of BE buffer directly to the center of the membrane.
16. Incubate at room temperature for 5 minutes.
17. Centrifuge at 8,000 rpm for 1 minute to elute DNA. Store the eluted DNA at –20 °C.

C. Urine DNA Extraction Protocol:

1. Transfer 1000 µL of urine sample into a 1.5 mL microcentrifuge tube and centrifuge at 10,000 rpm for 8 minutes.
2. Carefully discard 900 µL of the supernatant, then add 500 µL CL Buffer and vortex thoroughly.
3. Add Proteinase K to a final concentration of 0.4 mg/mL and vortex well. *(Proteinase K is not supplied with this kit; for example, add 10 µL of 20 mg/mL Proteinase K to 500 µL lysis buffer.)*
4. Incubate at 65 °C for 15 minutes.
5. Invert the tube several times during incubation to ensure proper mixing.
6. Centrifuge at 6,000 rpm for 10 minutes and transfer 400 µL of lysate to a new 1.5 mL tube.
7. Add 400 µL of BB buffer and vortex thoroughly.
8. Transfer the mixture to a spin column placed in a collection tube.
9. Centrifuge at 8,000 rpm for 1 minute. Discard the flow-through and place the column back into the collection tube.
10. If the sample volume exceeds the column capacity, repeat steps 8–9 until all the lysate has been processed.
11. Add 500 µL of BW buffer to the spin column and centrifuge at 10,000 rpm for 1 minute. Discard the flow-through.
12. Repeat the wash step once more with BW buffer.
13. Perform a dry spin at 10,000 rpm for 2 minute to remove residual buffer.
14. Transfer the spin column to a new 1.5 mL microcentrifuge tube.
15. Add 50 µL of BE buffer directly to the center of the membrane.
16. Incubate at room temperature for 5 minutes.
17. Centrifuge at 8,000 rpm for 1 minute to elute DNA. Store the eluted DNA at –20 °C.

D. Vaginal swab DNA Extraction Protocol:

1. Immediately after collection, place each swab into a sterile tube containing transport medium (e.g., PBS, TE buffer, or a suitable stabilization solution). Vortex thoroughly for 2 minutes to release the sample.
2. Transfer 500 µL of the medium into a 1.5 mL microcentrifuge tube and centrifuge at 10,000 rpm for 5 minutes.
3. Carefully remove 400 µL of the supernatant. Add 500 µL of CL Buffer and vortex thoroughly to resuspend the pellet.
4. Add Proteinase K to a final concentration of 0.4 mg/mL and vortex well. *(Proteinase K is not supplied with this kit; for example, add 10 µL of 20 mg/mL Proteinase K to 500 µL lysis buffer.)*
5. Incubate at 65 °C for 15 minutes.
6. Invert the tube several times during incubation to ensure proper mixing.
7. Centrifuge at 6,000 rpm for 10 minutes and transfer 400 µL of lysate to a new 1.5 mL tube.
8. Add 400 µL of BB buffer and vortex thoroughly.
9. Transfer the mixture to a spin column placed in a collection tube.
10. Centrifuge at 8,000 rpm for 1 minute. Discard the flow-through and place the column back into the collection tube.
11. If the sample volume exceeds the column capacity, repeat steps 9–10 until all the lysate has been processed.
12. Add 500 µL of BW buffer to the spin column and centrifuge at 10,000 rpm for 1 minute. Discard the flow-through.
13. Repeat the wash step once more with BW buffer.
14. Perform a dry spin at 10,000 rpm for 2 minutes to remove residual buffer.
15. Transfer the spin column to a new 1.5 mL microcentrifuge tube.
16. Add 50 µL of BE buffer directly to the center of the membrane.
17. Incubate at room temperature for 5 minutes.
18. Centrifuge at 8,000 rpm for 1 minute to elute DNA. Store the eluted DNA at –20 °C.

E. Faecal DNA Extraction Protocol:

1. Transfer up to 100 mg of faecal sample into a 1.5 mL microcentrifuge tube. Add 600 µL CL Buffer and vortex thoroughly.
2. Add Proteinase K to a final concentration of 0.3 mg/mL and vortex well. *(Proteinase K is not supplied with this kit; for example, add 9 µL of 20 mg/mL Proteinase K to 600 µL lysis buffer.)*
3. Incubate at 65 °C for 20 minutes.
4. During incubation, invert the tube several times to ensure proper mixing.
5. Centrifuge at 7,000 rpm for 10 minutes and transfer 400 µL of the clear lysate to a new 1.5 mL tube.
6. Add 400 µL of BB Buffer and vortex thoroughly.
7. Transfer the mixture to a spin column placed in a collection tube.
8. Centrifuge at 8,000 rpm for 1 minute. Discard the flow-through and place the column back into the collection tube.
9. If the sample volume exceeds the column capacity, repeat steps 7–8 until all the lysate has been processed.
10. Add 500 µL of BW Buffer to the spin column and centrifuge at 10,000 rpm for 1 minute. Discard the flow-through.
11. Repeat the wash step once more with BW Buffer.
12. Perform a dry spin at 10,000 rpm for 2 minutes to remove residual buffer.
13. Transfer the spin column to a new 1.5 mL microcentrifuge tube.
14. Add 50 µL of BE Buffer directly to the center of the membrane.
15. Incubate at room temperature for 5 minutes.
16. Centrifuge at 8,000 rpm for 1 minute to elute DNA. Store the eluted DNA at –20 °C.

F. Tissue DNA Extraction Protocol:

1. Transfer up to 50 mg of tissue into a 1.5 mL microcentrifuge tube. Add 600 µL CL Buffer and homogenize thoroughly.
2. Add Proteinase K to a final concentration of 0.4 mg/mL and vortex well. *(Proteinase K is not supplied with this kit; for example, add 12 µL of 20 mg/mL Proteinase K to 600 µL lysis buffer.)*
3. Incubate at 65 °C for 20 minutes.
4. During incubation, invert the tube several times to ensure proper mixing.
5. Centrifuge at 6,000 rpm for 10 minutes and transfer 400 µL of the clear lysate to a new 1.5 mL tube.
6. Add 400 µL of BB Buffer and vortex thoroughly.
7. Transfer the mixture to a spin column placed in a collection tube.
8. Centrifuge at 8,000 rpm for 1 minute. Discard the flow-through and place the column back into the collection tube.
9. If the sample volume exceeds the column capacity, repeat steps 7–8 until all the lysate has been processed.
10. Add 500 µL of BW Buffer to the spin column and centrifuge at 10,000 rpm for 1 minute. Discard the flow-through.
11. Repeat the wash step once more with BW Buffer.
12. Perform a dry spin at 10,000 rpm for 2 minutes to remove residual buffer.
13. Transfer the spin column to a new 1.5 mL microcentrifuge tube.
14. Add 50 µL of BE Buffer directly to the center of the membrane.
15. Incubate at room temperature for 5 minutes.
16. Centrifuge at 8,000 rpm for 1 minute to elute DNA. Store the eluted DNA at –20 °C.

Troubleshooting Guide:

Problem Observed	Possible Cause	Recommended Solution
Low DNA yield	Incomplete sample lysis	Ensure thorough vortexing or homogenization during the lysis step. Verify correct incubation temperature and time. Increase incubation time by 5–10 minutes for difficult samples (faecal or tissue).
	Insufficient Proteinase K activity	Confirm correct Proteinase K concentration and that the enzyme is active. Avoid repeated freeze–thaw cycles of Proteinase K.
	Low starting material	Increase input sample volume or mass within recommended limits.
	Inefficient DNA binding	Ensure correct volume of BB Buffer is added and mixed thoroughly before loading onto the column.
Poor DNA purity (A260/280 or A260/230 low)	Incomplete wash	Ensure proper BW Buffer washing steps are followed. Repeat wash if necessary.
	Residual ethanol carryover	Perform an additional dry spin (1–2 min) to remove residual wash buffer.
	Carryover of inhibitors (especially faecal samples)	Carefully transfer only clear lysate after centrifugation. Avoid disturbing debris or pellet.
DNA does not amplify in PCR/qPCR	Presence of PCR inhibitors	Perform an additional BW wash if inhibitors are suspected. Dilute the eluted DNA (1:5 or 1:10) before PCR.
	Low DNA concentration	Quantify DNA before downstream use. Increase elution efficiency by warming BE Buffer to 55–60 °C before elution.
Column clogging / slow flow-through	High particulate content	Ensure proper centrifugation and transfer only clear lysate.
	Sample too viscous	Increase lysis time or dilute lysate slightly before loading.
Viscous lysate	High cellular or protein content	Extend Proteinase K digestion time. For blood or tissue samples, mix more frequently during incubation.
Variable yields between samples	Inconsistent sample processing	Standardize sample input volume/mass and ensure uniform vortexing, incubation, and centrifugation conditions across samples.

DNA degradation	Prolonged storage of samples before extraction	Process samples promptly or store appropriately (e.g., blood at 4 °C short-term, −20 °C long-term). Avoid repeated freeze–thaw cycles.