SPINeasy™ DNA Kit for Tissue (With Lysing Matrix)

Cat. No.: 116558050 (50 PREPS) / 116558000 (5 PREPS)

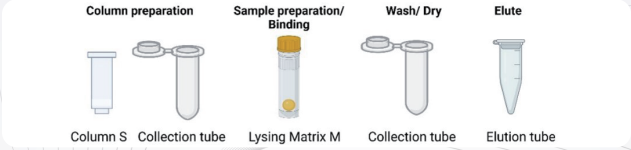
Quick-Start Protocol

Revision Nov 2023

Scan QR code for more information from instruction manual

Notes before starting:

- Add 12 mL (1.2 mL for sample kit) of absolute ethanol into **Buffer TD3** and mark the bottle.
- Add 50 mL (5 mL for sample kit) of absolute ethanol into **Buffer TD4** and mark the bottle.
- This kit requires the use of a centrifuge capable of generating at least 14,000 *g* to obtain optimal results. Use the maximum speed available if 14,000 *g* is not feasible.
- If FastPrep-24™ 5G (Cat. No.116005500) is not available, the use of a vortex capable of achieving 2,500 rpm is required.
- This Kit requires the use of a ThermoMixer capable of simultaneous shaking at 1,000 rpm and heating the samples to 56 ˚C.
- This kit can also be used with a vacuum manifold for the bind and wash step. Please refer to the instruction manual for more details.



Column preparation

***Optional:* Column preparation:**

Note: *Column preparation is recommended when higher DNA yield is desired or when column performance is reduced after long-term storage.*

1. *Pipette **200 µL Equilibration Buffer** into **Column S** (assembled with **Collection tube**). Incubate for **1 min** at room temperature and centrifuge the column for **30 sec @ 14,000 g**.*
2. *Keep the columns aside for later use (The treated Columns S can be stored at 2-8 ˚C for up to 7 days, if required).*

Sample preparation

DNA isolation protocol:

1. Weigh tissue (up to 10 mg for spleen tissue, up to 30 mg for other tissues) and place in a **Lysing Matrix M** tube.
2. Add **200 µL Buffer TD1, 20 µL Proteinase K** and **4 µL RNase A** into the tissue sample tube, vortex for **5 sec** to mix well. Briefly spin down the mixture.
3. Homogenize using FastPrep® for **5 sec @ 4 m/s** or vortex for **5 min @ 2,500 rpm**. Briefly spin down the lysate.
4. Incubate in a ThermoMixer at **1,000 rpm** for **10 min** at 56 ˚C. Briefly spin down the lysate.

Bind

5. Add **500 µL Buffer TD2** into the lysate. Mix thoroughly by pipetting up and down for **10 times** or vortex for **10 sec**. Briefly spin down the mixture.
6. Assemble Column S onto a clean Collection tube.
7. Load all the mixture (**~700 µL**) into Column S. Centrifuge for **30 sec @ 14,000 *g***. Discard flow through and place the column back into the same Collection tube.

Wash

8. Add **500 µL Buffer TD3** onto the center of the column, centrifuge for **30 sec @ 14,000 *g***. Discard flow through and place the column back into the same Collection tube.
9. Add **500 µL Buffer TD4** onto the center of the column, centrifuge for **30 sec @ 14,000 *g***. Discard flow through and place the column back into the same Collection tube (**Repeat this step once**).
10. Transfer the column to a **new** Collection tube and spin for **2 min @ maximum speed**.

Elute

11. Transfer the column to a **Elution tube**. Add **50-100 µL Buffer TD5** onto the center of the column, wait for **2 min** and centrifuge for **2 min @ 14,000 *g***. Purified DNA is now ready for downstream applications.

    Optional: *Perform a second elution step with further **50-100 µL Buffer TD5** will increase yields by up to 20%.*

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Flow-Chart

Column preparation

Optional:
Incubate 1 min at room temperature
14,000 g, 30 sec

Equilibration Buffer 200 µL

Sample preparation

Weigh tissue samples and add them into a tube of Lysing Matrix M

Buffer TD1 200 µL
Proteinase K 20 µL
RNase A 4 µL
Mix well
Quick spin

4 m/s
5 sec

Or

2,500 rpm
5 min
Quick spin

56 °C, 1,000 rpm, 10 min
Quick spin

Bind

Buffer TD2 500 µL
Mix well
Quick spin

Load all the mixture into Column S with Collection tube
14,000 g, 30 sec

Wash

Buffer TD3 500 µL
14,000 g, 30 sec

Repeat once

Buffer TD4 500 µL
14,000 g, 30 sec

Transfer the column to a new Collection tube

maximum speed, 2 min
Column drying

Elute

Transfer the column to Elution tube

Buffer TD5 50-100 µL
Incubate at RT for 2 min
14,000 g, 2 min

Highly purified genomic DNA

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