SPINeasy DNA Kit for Tissue and Bacteria (With Lysing Matrix)

Cat. No.: 116532050 (50 preps) & 116532005 (5 preps)

Quick-Start Protocol

Revision 1.0 (March 2021)

Notes before starting:

- ☐ Add 50 mL (5 mL for sample kit) of absolute ethanol to Wash Buffer GD2 and mark on the bottle.
- ☐ Prepare one 1.5 mL microcentrifuge tube per prep for elution of purified genomic DNA.
- ☐ Lysis can be performed by vortexing the sample in a vial of Lysing Matrix A at the maximum speed if a FastPrep® Instrument is unavailable.
- ☐ Centrifugation speed stated in the manual will be a guideline; use the maximum speed available if 14,000 x g is not feasible.
- ☐ Only required for DNA extraction from gram-positive bacteria: prepare pre-treatment buffer according to instructions on the back of this protocol (to be supplied by user).

Lyse

1. Refer to back of this protocol for summary of sample preparation in **Lysing Matrix A**.
2. Homogenize in a FastPrep® Instrument for 15 seconds at speed setting of 4.0 m/s.

   Alternatively, vortex samples at the maximum speed for 20 mins for animal tissues and bacteria or 10 mins for blood and cultured cells.
3. Centrifuge at 14,000 x g for 10 mins.

Bind

4. Transfer 750 µL of the supernatant to a **Column GD with collection tube.**
5. Centrifuge at 14,000 x g for 1 min. Discard flow through and reuse collection tube.

Wash

6. Add 500 µL of **Wash Buffer GD1** to the column.
7. Centrifuge at 14,000 x g for 1 min. Discard flow through and reuse collection tube.
8. Add 750 µL of **Wash Buffer GD2** to the column. Incubate at room temperature for 1 min.
9. Centrifuge at 14,000 x g for 1 min. Discard flow through and reuse collection tube.
10. Centrifuge at 14,000 x g for an additional 1 min to dry column.
11. Optional: Incubate at 55°C for 3 – 5 mins to dry column completely.

Elute

12. Remove collection tube and place column onto a clean 1.5 mL microcentrifuge tube.
13. Add 100 µL of **Elution Buffer GD** to the center of the membrane. Incubate at room temperature for 1 min.
14. Centrifuge at 8,000 x g for 1 – 2 mins to elute DNA.
15. Eluted genomic DNA will be collected in the microcentrifuge tube.

Summary of sample preparation in Lysing Matrix A

Sample type	Starting amount	Lysis Buffer GD	Procedure
Animal tissues	≤30 mg (≤10 mg for spleen)	1 mL	Cut tissue into small pieces, transfer to **Lysing Matrix A** and add 1 mL **Lysis Buffer GD.**
Blood (non-nucleated)	100 µL – 200 µL	Make up to 1 mL final volume	Add **Lysis Buffer GD** to make up to 1 mL final volume, mix and transfer the mixture into a vial of **Lysing Matrix A.**
Blood (nucleated)	10 µL	Make up to 1 mL final volume	Add **Lysis Buffer GD** to make up to 1 mL final volume, mix and transfer the mixture into a vial of **Lysing Matrix A.**
Cultured cell pellet	1 x 10^6 cells (up to 5 x 10^6 cells)	1 mL	Resuspend cell pellet in 1 mL of **Lysis buffer GD** and transfer to a vial of **Lysing Matrix A.**
Cultured cells in 100 µL PBS	1 x 10^6 cells (up to 5 x 10^6 cells)	900 µL	Add **Lysis Buffer GD** to make up to 1 mL final volume, mix and transfer the mixture into a vial of **Lysing Matrix A.**
Gram-negative bacteria	Pellet from 3 mL to 9 mL of overnight culture	1 mL	Resuspend cell pellet in 1 mL of **Lysis buffer GD** and transfer to a vial of **Lysing Matrix A.**
Gram-positive bacteria	Pellet from 3 mL to 9 mL of overnight culture	800 µL, following pre-treatment	Perform pre-treatment as below, add 800µL of **Lysis Buffer GD**, mix and transfer to a vial of **Lysing Matrix A**.

Pre-treatment of Gram-positive bacteria

1. Prepare gram-positive bacteria pre-treatment buffer (to be supplied by user) containing:
   20 mM Tris-HCl, pH 8.0
   2 mM EDTA, pH 8.0
   1.2% Triton® X-100
   20 mg/mL lysozyme. Store at 4°C after adding lysozyme.
2. Resuspend bacterial pellet in 200 µL of gram-positive bacteria pre-treatment buffer.
3. Incubate for 30 mins at 37°C.
4. Add 800 µL of Lysis Buffer GD, mix and transfer to a vial of Lysing Matrix A. Proceed to step 2 of the Quick-Start Protocol.

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