SPINeasy™ DNA/RNA/Protein All-In-One Kit

Cat. No.: 116544050 (50 PREPS) / 116544000 (5 PREPS)

Quick-Start Protocol

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Notes before starting:

- Add 100 mL (10 mL for sample kit) of absolute ethanol to Wash Buffer R and mark the bottle.
- Prepare DNase I solution by spinning down briefly the vial of lyophilized DNase I provided, resuspend and dissolve with 500 µL **Nuclease-free water**. Store DNase I solution at -20 °C in aliquots and avoid repeated freeze-thawing. Do not prepare DNase I solution in DNase I buffer.
- Prepare 750 µL of ethanol per prep for RNA binding.
- Prepare 500 µL of 50% ethanol per prep for washing of protein pellet.
- Prepare 100–200 µL of the buffer of choice* per prep for resuspension of protein pellet. *Refer to Step 32.
- Use maximum centrifugation speed available if 14,000 *g* is not feasible.
- For some samples, lysis may be performed by vortexing the sample in a vial of Lysing Matrix A at the maximum speed for 3–5 min if a FastPrep® Instrument is unavailable.

Column preparation

1. Add **200 µL Equilibration Buffer** to **Column A with collection tube** membrane to ensure its performance. Centrifuge for **1 min @ maximum speed.** Discard the flow-through and reuse the collection tube.

Lyse

2. Weigh **10–30 mg** of animal tissue. Cut tissue into small pieces, transfer to a vial of **Lysing Matrix A** and add **1 mL Lysis Buffer R.**

   Cell culture: Resuspend cell pellet (1x10^6 cells recommended) in **1 mL Lysis Buffer R** and transfer to a vial of **Lysing Matrix A.**
3. Homogenize in a FastPrep® Instrument for **15 sec** at speed setting of **4 m/s**.

DNA extraction

4. Centrifuge for **10 min @ 14,000 *g*** and transfer the supernatant (~750 µL) to a **Column A with collection tube.**
5. Centrifuge for **1 min @ 14,000 *g***.
6. Transfer the flow-through to a clean 2 mL microcentrifuge tube and place the column back onto the **same** collection tube. The flow-through will be used for RNA extraction (Step 12).
7. Add **500 µL Wash Buffer D** to the column. Centrifuge for **1 min @ 14,000 *g***. Discard flow-through and reuse collection tube.
8. Add **500 µL Wash Buffer R** to the column. Centrifuge for **1 min @ 14,000 *g***. Discard flow-through and reuse collection tube.
9. Centrifuge for an additional **1 min @ 14,000 *g*** to dry column.
10. Remove collection tube and place column onto a clean 1.5 mL microcentrifuge tube. Add **100 µL Elution Buffer GD** to the center of the membrane. Incubate at room temperature for **1 min**. For samples with low DNA content, reducing the elution volume to **50 µL** may increase the concentration of eluted DNA.
11. Centrifuge for **1–2 min @ 8,000 *g*** to elute DNA. Store DNA at -20 °C.

RNA extraction

12. Add **750 µL** absolute ethanol to the flow-through collected in Step 6. Mix well by pipetting up and down.
13. Transfer 750 µL of the mixture to a **new Column A with collection tube**.
14. Centrifuge for **1 min @ 14,000 *g***.
15. Transfer the flow-through to a clean 2 mL microcentrifuge tube and place column back onto the **same** collection tube. The flow-through will be used for protein extraction (Step 25).
16. Repeat steps 13–15 to load the remaining mixture in the **same** column and collect all the flow-through for protein extraction.
17. Add **500 µL Wash Buffer R** to the column. Centrifuge for **1 min @ 14,000 *g***. Discard flow-through and reuse collection tube.
18. DNase I digestion: In a clean 1.5 mL microcentrifuge tube, add **5 µL** DNase I solution to **75 µL DNase I Buffer** per prep. Mix well and add **80 µL** to the center of the column membrane. Incubate at room temperature for **15 min.**
19. Add **500 µL Wash Buffer R** to the column. Centrifuge for **1 min @ 14,000 *g***. Discard flow-through and reuse collection tube.
20. Repeat Step 19 to wash a second time.
21. Centrifuge for **an additional 1 min @ 14,000 *g*** to dry column.
22. Remove collection tube and place column onto a clean 1.5 mL microcentrifuge tube.
23. Add **100 µL Nuclease-free water** to the center of the membrane. Incubate at room temperature for **1 min**. For samples with low RNA content, reducing the elution volume to **50 µL** may increase the concentration of eluted RNA.
24. Centrifuge for **1–2 min @ 8,000 *g*** to elute RNA.

    Eluted RNA will be collected in the microcentrifuge tube. For the best results, proceed to perform downstream applications immediately and keep RNA chilled on ice while working to prevent degradation. Store remaining RNA at -80 °C in aliquots and avoid repeated freeze-thawing.

Protein extraction

25. Add an equal volume of **Protein Precipitant** to the flow-through collected in Steps 15 and 16.
26. Mix well by inverting tubes several times and incubate at room temperature for at least **10 min** to allow protein precipitation.
27. Centrifuge for **10 min @ 14,000 *g***. Carefully discard supernatant.
28. Add **500 µL** of 50% ethanol and invert tubes several times to wash pellet.
29. Centrifuge for **2 min @ 14,000 *g***.
30. Carefully remove supernatant, leaving the protein pellet in the tube.
31. Dry pellet by uncapping the tube and leaving it at room temperature for **10 min** or longer.
32. Resuspend pellet with **100–200 µL** of the buffer of choice*, depending on the intended downstream application.

    *For convenience, pellet may be dissolved in protein loading dyes, such as Laemmli buffer, and directly used for SDS-PAGE applications. Alternatively, pellet may be dissolved in 5% SDS solution. Note that SDS interferes with certain protein quantitation methods, such as the Bradford assay. Thus, buffers containing SDS, including protein loading buffers, are incompatible with such applications. If protein quantitation is necessary, the pellet may be dissolved in 50 mM NaOH, followed by neutralization with an equal volume of 50 mM HCl.

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