SPINeasy RNA Kit for Tissue (With Lysing Matrix)

Cat. No.: 116543050 (50 preps) & 116543000 (5 preps)

Please refer to the next page for SPINeasy RNA Kit for Tissue (Without Lysing Matrix)

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Quick-Start Protocol

Revision 1.0 (September 2021)

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 of **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.
- ☐ Use maximum centrifugation speed available if 14,000 x 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.

Lyse

1. Animal/ plant tissue: Weigh 10 – 30 mg of animal tissue or 50 – 100 mg of plant 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 (1 x 10^6 cells recommended) in 1 mL **Lysis Buffer R** and transfer to a vial of **Lysing Matrix A**.
2. Homogenize in a FastPrep Instrument for 15 seconds at speed setting of 4.0 m/s

Bind

3. Centrifuge at 14,000 x g for 10 min and transfer lysate supernatant to a clean, nuclease-free 2 mL microcentrifuge tube.
4. Add equal volume of absolute ethanol and mix well by pipetting up and down.
5. Transfer 750 µL of the mixture to a **Column R with collection tube**.
6. Centrifuge at 14,000 x g for 1 min. Discard flow through and reuse collection tube. Repeat steps 5 and 6 to load the remaining mixture.

Wash

7. Add 500 µL of **Wash Buffer R** to the column.
8. Centrifuge at 14,000 x g for 1 min. Discard flow through and reuse collection tube.
9. DNase I digestion: In a clean 1.5 mL microcentrifuge tube, add 5 µL of DNase I solution to 75 µL of **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.
10. Add 500 µL of **Wash Buffer R** to the column.
11. Centrifuge at 14,000 x g for 1 min. Discard flow through and reuse collection tube. Repeat steps 10 and 11 to wash a second time.

Dry

12. Centrifuge at 14,000 x g for an additional 1 min to dry column. Remove collection tube and place column onto a clean 1.5 mL microcentrifuge tube.

Elute

13. Add 100 µL of **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.
14. Centrifuge at 8,000 x g for 1 – 2 mins to elute RNA.
15. 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.

SPINeasy RNA Kit for Tissue (Without Lysing Matrix)

Cat. No.: 116542050 (50 preps)

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Quick-Start Protocol

Revision 1.0 (September 2021)

Notes before starting:

- ☐ Add 100 mL 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 of **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.
- ☐ Use maximum centrifugation speed available if 14,000 x g is not feasible.

Lyse

1. Animal/ plant tissue: Weigh 10 – 30 mg of animal tissue or 50 – 100 mg of plant tissue. Homogenize tissue using a mechanical lysis method, such as homogenizer or grind tissue in liquid nitrogen and transfer to a clean, nuclease-free 1.5 mL microcentrifuge tube. Add 1 mL **Lysis Buffer R** and mix by vortexing.
   Cell culture: Resuspend cell pellet (1×10^6 cells recommended) in 750 µL of **Lysis Buffer R** and transfer to a clean, nuclease-free 1.5 mL microcentrifuge tube. Vortex for 1 min and incubate at 55°C for 10 mins. Vortex for 1 min after the incubation.
2. Centrifuge at 14,000 x g for 5 min
3. Transfer lysate supernatant to a clean, nuclease-free 2 mL microcentrifuge tube.

Bind

4. Add equal volume of absolute ethanol (e.g., 750 µL ethanol to 750 µL lysate supernatant) and mix well by pipetting up and down.
5. Transfer 750 µL of the mixture to a **Column R with collection tube**.
6. Centrifuge at 14,000 x g for 1 min. Discard flow through and reuse collection tube.
7. Repeat steps 5 and 6 to load the remaining mixture.

Wash

8. Add 500 µL of **Wash Buffer R** to the column.
9. Centrifuge at 14,000 x g for 1 min. Discard flow through and reuse collection tube.
10. DNase I digestion: In a clean 1.5 mL microcentrifuge tube, add 5 µL of DNase I solution to 75 µL of **DNase I buffer** per prep. Mix well and add 80 µL to the center of the column membrane.Incubate at room temperature for 15 mins.
11. Add 500 µL of **Wash Buffer R** to the column.
12. Centrifuge at 14,000 x g for 1 min. Discard flow through and reuse collection tube.
13. Repeat steps 11 and 12 to wash a second time.

Dry

14. Centrifuge at 14,000 x g for an additional 1 min to dry column. Remove collection tube and place column onto a clean 1.5 mL microcentrifuge tube.

Elute

15. Add 100 µL of **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.
16. Centrifuge at 8,000 x g for 1 – 2 mins to elute RNA.
17. 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.