SPINeasy™ RNA Kit for Yeast

Cat. No.: 116565050 (50 PREPS) / 116565000 (5 PREPS)

Quick-Start Protocol

Revision Oct 2023

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This protocol is designed to extract RNA from Yeast sample.

Notes before starting:

- Materials to be supplied by user: **β-Mercaptoethanol and ethanol (96-100%).**
- This protocol requires a centrifuge capable of generating at least 15,000 *g* to obtain optimal results.
- Add 2 mL isopropanol to Binding Buffer BB.
- Add 91 mL ethanol to Wash Buffer Y2.

Column preparation

1. Add **200 µL Equilibration Buffer** into the **DNA Removal Column** (placed in **2.0 mL Collection Tube**) and **RNA Column**.
2. Wait at least **1 min** and centrifuge for **10 sec @ maximum speed**.
3. Transfer the columns into new **2.0 mL Collection Tube** and **RNA Collection Tube** (provided).

Sample preparation

4. Transfer the yeast cell culture (up to **2 x 10^8** yeast cells) to a 2 mL microcentrifuge tube (not provided). Centrifuge the cells for **3-5 min @ 15,000 *g***, discard the supernatant.
5. Resuspend the cell pellet with **750 µL Lysis Buffer LB, 20 µL Proteinase K** and **15 µL β-Mercaptoethanol (β-Mer)** (not provided) and then transfer all lysate to a **Lysing Matrix YG** tube.
6. Homogenize using vortex for **20 min @ 2,500 rpm** or Fastprep® for **40 sec @ 6m/sec**, then centrifuge for **2 min @ 15,000 *g*** and transfer the supernatant **(~630 µL)** to a **2 mL Tube** (provided).

DNA removal

7. Add **160 µL Binding Buffer BB** to the supernatant from the above step, vortex for **30 sec**, then centrifuge for **2 min @ 15,000 *g***.
8. Transfer the clear supernatant **(~650 µL)** to the pre-treated DNA Removal Column and centrifuge for **20 sec @ 15,000 *g*** to bind/remove the genomic DNA from the lysate. Discard the column and remain the flowthrough for the next step.

RNA binding

9. To the flowthrough from the above step, add **1 volume of ethanol**. For example, add **650 µL** ethanol to **650 µL** supernatant.
10. Load **700 µL** of the above mixture into the RNA Column and centrifuge for **20 sec @ 15,000 *g***.
11. Repeat until all the above mixture has been transferred to the RNA Column.

RNA washing

12. Add **700 µL Wash Buffer Y2** to the RNA Column and centrifuge for **20 sec @ 15,000 *g***. Repeat the above wash again.
13. Transfer the RNA Column to a new **RNA Collection Tube** (provided) and centrifuge for **1 min @ maximum speed** to remove any residual ethanol.

Elute

14. Place the RNA Column in a new 1.5 mL **Elution Tube** (provided). Add **100 µL RNase Free Water** to the center of the column membrane, wait for **1 min**, and then centrifuge for **1 min @ maximum speed**.
15. The RNA samples are now ready for downstream processing.

Note: *Eluting with **100 µL RNase Free Water** will maximize nucleic acid yield. For a more concentrated sample, a minimum of **50 µL RNase Free Water** can be used.*

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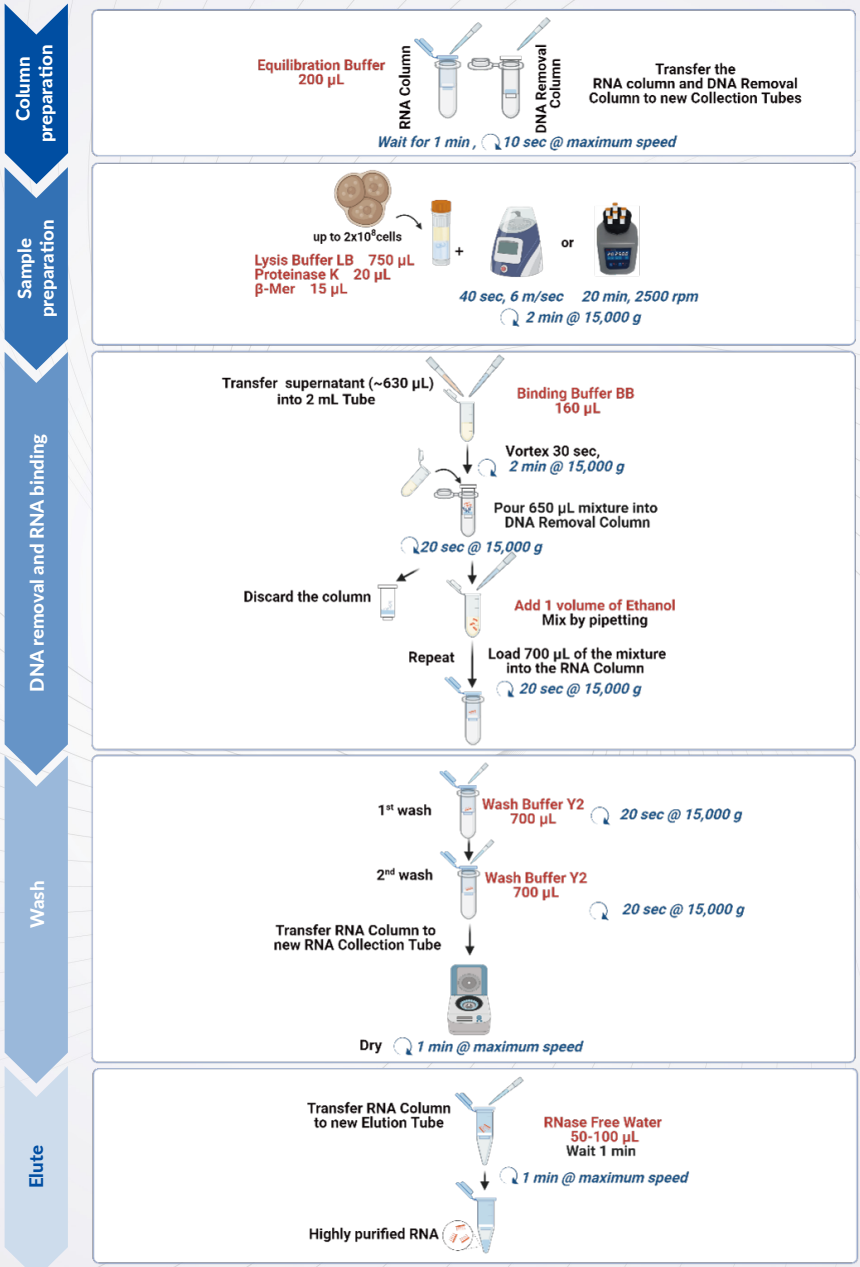
Flow-Chart



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