

SPINeasy™ RNA Kit for Yeast

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



Quick-Start Protocol

Revision Oct 2023

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.



Scan QR code for more information from instruction manual

Column preparation

1. Add **200 μ L 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 **2×10^8** 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 μ L Lysis Buffer LB**, **20 μ L Proteinase K** and **15 μ L 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 (**\sim 630 μ L**) to a **2 mL Tube** (provided).

DNA removal

7. Add **160 μ L 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 (**\sim 650 μ L**) 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 μ L ethanol to 650 μ L supernatant**.
10. Load **700 μ L** 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 μ L 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 μ L 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 μ L RNase Free Water** will maximize nucleic acid yield. For a more concentrated sample, a minimum of **50 μ L RNase Free Water** can be used.

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Flow-Chart

Column preparation

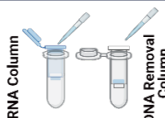
Sample preparation

DNA removal and RNA binding

Wash

Elute

Equilibration Buffer
200 μ L



Transfer the RNA column and DNA Removal Column to new Collection Tubes

Wait for 1 min, \curvearrowright 10 sec @ maximum speed

up to 2×10^8 cells
Lysis Buffer LB 750 μ L
Proteinase K 20 μ L
 β -Mer 15 μ L



40 sec, 6 m/sec or 20 min, 2500 rpm
 \curvearrowright 2 min @ 15,000 g

Transfer supernatant (~630 μ L) into 2 mL Tube

Binding Buffer BB
160 μ L



Vortex 30 sec,
2 min @ 15,000 g

Pour 650 μ L mixture into
DNA Removal Column

\curvearrowright 20 sec @ 15,000 g

Discard the column

Add 1 volume of Ethanol
Mix by pipetting

Repeat

Load 700 μ L of the mixture into the RNA Column
 \curvearrowright 20 sec @ 15,000 g

1st wash Wash Buffer Y2 700 μ L \curvearrowright 20 sec @ 15,000 g

2nd wash Wash Buffer Y2 700 μ L \curvearrowright 20 sec @ 15,000 g

Transfer RNA Column to new RNA Collection Tube

Dry \curvearrowright 1 min @ maximum speed

Transfer RNA Column to new Elution Tube

RNase Free Water
50-100 μ L
Wait 1 min

\curvearrowright 1 min @ maximum speed

Highly purified RNA



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