

SPINeasy™ DNA Kit for Yeast

Cat. No.: 116557050 (50 PREPS) / 116557000 (5 PREPS)



Quick-Start Protocol

Revision Oct 2023

Notes before starting:

- This protocol requires a centrifuge capable of generating at least 15,000 g to obtain optimal results.
- **Wash Buffer Y2** is supplied as a concentrate. Before using for the first time, add absolute alcohol as indicated on the bottle to obtain a working solution.



Scan QR code for more information from instruction manual

Column preparation

- Add **200 µL Equilibration Buffer** into the **Column S** placed in **Collection tube** (provided), centrifuge for **1 min @ 15,000 g**. Discard the flow-through and reuse Collection tube.

Sample preparation

- Harvesting cells from liquid culture
Pellet the cells 1-2 mL (maximum 4×10^8 cells) by centrifuging for **5 min @ 15,000 g**. Discard the supernatant.
- Harvesting colonies from solid medium
Scrape a single colony (≥ 2 mm in diameter) or amount **< 50 mg** from solid medium.

Lysis

1. Add **5 µL RNase A** and **560 µL Lysis Buffer Y** to each microcentrifuge tube of pellet collected. Re-suspend the pellet thoroughly by pipetting repeatedly. Transfer all lysate to **Lysing Matrix YG** tube.
2. Homogenize in a FastPrep® Instrument for **35 sec** at speed setting of **5.0 m/sec** or vortex for **10 min** at speed setting of **2,500 rpm**.
3. Centrifuge for **2 min @ 15,000 g**.
Note: Cells $> 4 \times 10^8$ or colonies ≥ 50 mg, homogenize twice.

Bind

4. Slowly add **560 µL Binding Buffer Y** into the Lysing Matrix YG tube, then mix thoroughly.
5. Centrifuge for **1 min @ 15,000 g**.
6. Carefully transfer **750 µL** of the supernatant to Column S.
7. Centrifuge for **1 min @ 15,000 g**.

Wash

8. Add **600 µL Wash Buffer Y1** to the wall of Column S. Centrifuge for **1 min @ 15,000 g**. Discard the flow-through.
9. Add **700 µL Wash Buffer Y2** to the wall of Column S. Centrifuge for **1 min @ 15,000 g**. Discard the flow-through.
10. Centrifuge Column S for **2 min @ 15,000 g** to dry the column.

Elute

11. Transfer the column into a clean **Elution tube** (provided). Add **50-200 µL Elution Buffer EB** or ultrapure water (**pH >7.0**) (not provided) to the center of column, wait for **2 min**, and centrifuge for **1 min @ 15,000 g**. The DNA samples are now ready for downstream application.

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
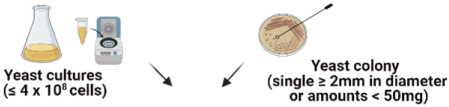



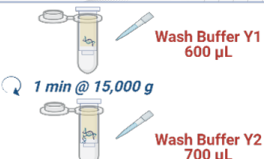



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Flow-Chart



 <p>Equilibration Buffer 200 µL</p>
<p>1 min @ 15,000 g</p> <p>Discard the flowthrough Reuse Collection Tube</p>
 <p>Yeast cultures ($\leq 4 \times 10^8$ cells)</p> <p>Yeast colony (single ≥ 2mm in diameter or amounts < 50mg)</p>
 <p>RNase A 5 µL</p> <p>Lysis Buffer Y 560 µL</p> <p>Resuspend pellet thoroughly by pipetting Transfer all lysate to Lysing Matrix YG tube</p>
 <p>35 sec, 5 m/sec</p> <p>10 min, 2500 rpm</p> <p>2 min @ 15,000 g</p>
 <p>Binding Buffer Y 560 µL</p> <p>Mix thoroughly 1 min @ 15,000 g</p> <p>Transfer 750 µL supernatant into Column S</p>
 <p>Wash Buffer Y1 600 µL</p> <p>Wash Buffer Y2 700 µL</p> <p>Dry Column</p>
 <p>Elution Buffer EB 50-200 µL</p> <p>Wait for 2 min</p> <p>1 min @ 15,000 g</p> <p>Highly purified DNA</p>



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