

Quick Start Guide

GenElute™ Direct mRNA Kit

DMN10, DMN70

Reagents to Prepare

- Dissolve the Proteinase K in 40% glycerol. Use 0.5 mL of 40% glycerol for 5 mg Proteinase K (10 preps) or 2.5 mL of 40% glycerol for 25 mg Proteinase K (70 preps). Mix thoroughly before use. Store at 2-8 °C.
- Prepare fresh Lysis Solution with Proteinase K by adding 20 µl of Proteinase K solution per mL of Lysis Solution and mixing thoroughly. The required amount per preparation is 0.5 mL for cultured cells and 1.0 mL for tissue.

Protocol

All spins at $\geq 12,000 \times g$.

Release RNA from Cells

1. Cultured Cells
 - a. Pellet up to 10^7 cells. Discard medium.
 - b. Add 0.5 ml Lysis Solution containing Proteinase K to cell pellet & vortex.
 - c. Transfer lysate to filtration column and spin 2 min.
 - d. Continue to **Digest Proteins and Bind mRNA to Beads.**

Release RNA from Tissues

1. Mammalian Tissues
 - a. Place up to 50 mg tissue in homogenizer vessel.
 - b. Add 1 ml Lysis Solution containing Proteinase K and homogenize immediately.
 - c. Continue to **Digest Proteins and Bind mRNA to Beads.**

Digest Proteins and Bind mRNA to Beads

2. Incubate lysate at 65 °C for 10 min.
3. Add 32 µl 5M NaCl to cells OR 64 µl 5M NaCl to tissue.
4. Add 25 µl oligo (dT) beads and vortex.
5. Incubate at room temperature for 10 min.
6. Spin 5 min to pellet beads. Discard all but ~50 µl supernatant.

Optional: Release mRNA & Rebind

7. Resuspend beads in 500 µl Lysis Solution + 32 µl 5M NaCl Pulse vortex.
8. Incubate at 65 °C for 5 min.
9. Incubate at room temperature for 5 min.
10. Spin for 2 min to pellet beads. Discard all but ~50 µl supernatant.

Wash to Remove Contaminants

11. Thoroughly resuspend beads in 350 µl Wash Solution and transfer to spin column.
12. Spin 1-2 min. Discard flow-through.
13. **2x:** Add 350 µl Low Salt Wash Solution. Spin 1-2 min. Discard flow-through.

Elute Purified mRNA

14. Transfer column to new collection tube.
15. Add 50 µl Elution Buffer (pre-warmed to 65°C).
16. Incubate at 65°C for 2-5 min. Spin 1 min.
Optional: Repeat elution for additional yield.

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