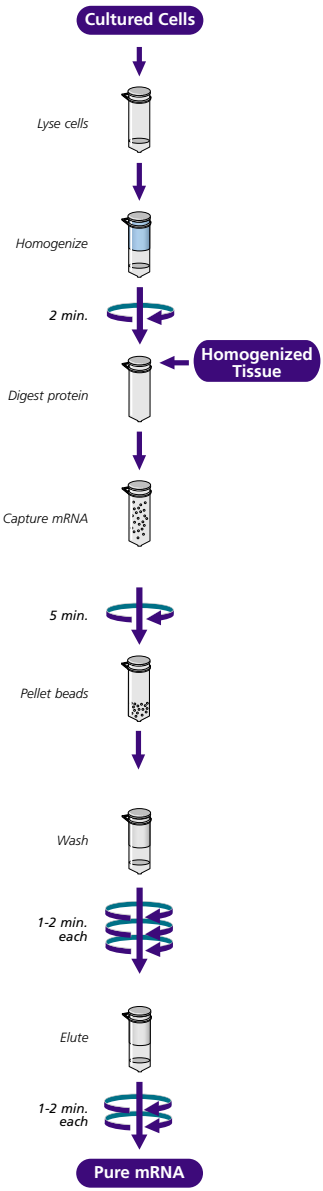
GENELUTE DIRECT mRNA MINIPREP KITS



All spins at maximum speed (16,000 x g)

1 Release RNA from cells or tissues

A. Cultured cells

- ❑ Pellet up to 10^7 cells. Discard medium.
- ❑ Mix 10 µl Proteinase K with 0.5 ml Lysis Solution. Add to cell pellet & vortex.
- ❑ Transfer lysate to Filtration Column & spin 2 min. Discard column.

B. Mammalian Tissues

- ❑ Mix 20 µl Proteinase K with 1.0 ml Lysis Solution.
- ❑ Place up to 50 mg tissue in homogenizer vessel.
- ❑ Add 1.0 ml Lysis Solution/Proteinase K & homogenize immediately.

2 Digest proteins & bind mRNA to oligo (dT) beads

- ❑ Incubate at 65°C for 10 minutes.
- ❑ Add 32 µl 5 M NaCl to cells OR 64 µl 5 M NaCl to tissue.
- ❑ Add 25 µl oligo (dT) beads. Vortex.
- ❑ Incubate at room temperature, 10 min.
- ❑ Pellet beads 5 min. Remove all but ~ 50 µl supernatant & discard.

3 Release mRNA & rebind *(optional)*

- ❑ Resuspend beads in 500 µL Lysis Solution + 32 µL 5 M NaCl
- ❑ Incubate at 65°C, 5 min.
- ❑ Incubate at room temperature, 5 min.
- ❑ Pellet beads 2 min. Remove all but ~ 50 µl supernatant & discard.

4 Wash to remove contaminants

- ❑ Resuspend beads in 350 µL Wash Solution & transfer to Spin Column.
- ❑ Spin 1-2 min. Discard flow-through.
- ❑ Add 350 µL Low Salt Wash Solution. Spin 1-2 min. Discard flow-through.
- ❑ Repeat Low Salt wash.

5 Elute purified mRNA

- ❑ Transfer column to new collection tube.
- ❑ Add 50 µl Elution Solution (pre-warmed to 65°C).
- ❑ Incubate at 65°C, 2-5 min. Spin 1-2 min.
- ❑ Repeat elution.

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Problem	Reason	Solution
Clogged spin column	Sample size was too large	For future preparations, use fewer cells or smaller tissue samples. Alternatively, undigested material may be pelleted for 2-5 minutes after the proteinase K digest in step 3, and the supenatant liquid transferred to a new tube before adding NaCl and oligo (dT) beads. To salvage the current preparation, spin longer than 1-2 minutes until solutions pass through the spin filter. Yield and purity of mRNA will likely be reduced.
	Homogenization was incomplete	Cell lysates must be spun through filtration columns to shear DNA. Tissues must be thoroughly homogenized until no visible particles remain.
	Digestion was incomplete	Store proteinase K at 2-8ºC after it is dissolved in glycerol. Add proteinase K to Lysis Solution immediately before use. The enzyme is not stable in Lysis Solution for extended times. Verify that homogenized cells or tissues were incubated at 65ºC for 10 min before NaCl was added.
	Centrifugal force was low	If the microcentrifuge used can not attain 16,000 x g, longer spin times may be required.
Low yield	Cells or tissue had low mRNA levels	Yields will vary greatly between different types of cells and tissues. See "Expected Yield" in the Technical Bulletin.
	Elution Solution was not pre-heated or samples were not incubated at 65ºC	Transfer ~ 120 µl of Elution Solution per preparation into a microcentrifuge tube & heat at 65ºC in heating block before starting the procedure. Incubate bead:mRNA complex with Elution Solution for 2-5 min at 65ºC before spinning.
Degraded mRNA	Tissue or culture was old	Use cultures before they reach maximum density or become fully confluent, and harvest tissues as rapidly as possible from freshly sacrificed animals.
	Cells or tissue were stored improperly	If immediate preparation of mRNA is not possible, flash-freeze cell pellets or small pieces of tissue in liquid nitrogen & store at -70ºC. Do not allow material to thaw until it is disrupted in Lysis Solution.
	Cells or tissue contained high levels of RNase	Cells such as monocytes and macrophages, and tissues such as pancreas, spleen, and thymus, are rich in RNases and require immediate and thorough disruption in Lysis Solution to prevent degradation of RNA.
	Cells or tissues were not disrupted sufficiently	Vortex or pipet cell lysates until no clumps remain. Homogenize tissues in lysis solution until no visible particles remain.
	Proteinase K digestion was incomplete	See "Digestion was incomplete" above.
	RNase was introduced during the procedure	Pay special attention to precautions for handling RNA samples and related lab equipment listed at the beginning of the Technical Bulletin & its references 2-4.
Excessive rRNA contamination	Abundance of rRNA is high; sequence of rRNA contains poly(A) regions	Detectable amounts of rRNA are expected. Non-specific binding to oligo (dT) will occur due to the vast excess of rRNA over mRNA. Also, poly A regions in rRNA can bind specifically to the beads. If a more enriched preparation is desired, re-purify the mRNA by adding Lysis Solution to 500 µl, 5 M NaCl to 0.5 M, & 25 µl oligo (dT) beads. Vortex, incubate 10 min, pellet beads, wash & elute as before.
	Mini-prep capacity was exceeded	Re-purify as above. For future preparations, use smaller amounts of starting cells or tissue.
	Releasing and rebinding procedures were omitted	Re-purify as above.
Poor results in downstream procedures	Salt carried over into eluate	Spin beads dry before adding Elution Solution.
	Improper storage or handling	Store eluted mRNA in elution buffer at -70ºC or as ethanol precipitate at -70ºC until needed. Keep the mRNA on ice whenever it is thawed for use.