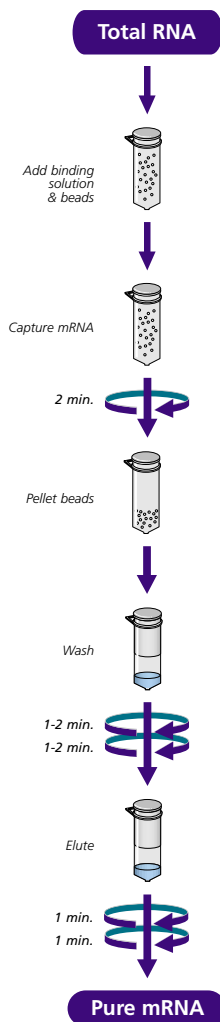


## GENELUTE mRNA MINIPREP KITS



All spins at max speed (14,000-16,000 x g)

### 1 Bind mRNA to oligo (dT) beads

- Bring volume of total RNA to 250  $\mu$ l with RNase-free water.
- Add 250  $\mu$ l 2x Binding Solution.
- Add 15  $\mu$ l beads. Vortex thoroughly.
- Incubate 3 minutes at 70°C.
- Place at room temperature for 10 minutes.
- Spin 2 minutes to pellet beads: mRNA complex. Remove supernatant.

### 2 Wash to remove contaminants

- Resuspend pellet in 500  $\mu$ l Wash Solution. Transfer to spin filter. Spin 1-2 minutes. Discard flow-through.
- Add second 500  $\mu$ l Wash Solution. Spin 1-2 minutes.

### 3 Elute purified mRNA

- Place spin filter into fresh receiving tube.
- Pipette 50  $\mu$ l Elution Buffer (pre-warmed to 70°C) into center of filter. Incubate at 70°C for 2-5 minutes. Spin 1 minute.
- Repeat elution using same collection tube.



## GENELUTE mRNA MINIPREP KITS

Problem	Reason	Solution
Low yield	RNA was derived from cells or tissue that had low mRNA level	Yields will vary greatly between different types of cells and tissues. See "Expected Yield" in Technical Bulletin.
	Start material is not pure	Ensure the starting total RNA does not contain any components (such as RNase) that would inhibit the proper isolation of poly(A) <sup>+</sup> mRNA during this procedure. We recommend using RNA isolation with GenElute Mammalian Total RNA Kits (Product Nos. RTN10, RTN70 and RTN350).
Degraded mRNA	Too much or too little starting material	Use 5-500 ug of total RNA /15 µl of oligo (dT) polystyrene beads.
	Elution Solution was not pre-heated or samples were not incubated at 70°C	Transfer ~ 120 µl of Elution Solution per preparation into a microcentrifuge tube & heat at 70°C in heating block before starting the procedure. Incubate bead:mRNA complex with Elution Solution for 2-5 min at 70°C before spinning.
Excessive rRNA contamination	Starting total RNA was degraded or contaminated	Be certain the procedure used to isolate the total RNA removes RNases. Check integrity of total RNA by agarose gel electrophoresis before preparing mRNA.
	Abundance of rRNA is high; sequence of rRNA contains poly(A) regions	Pay special attention to precautions for handling RNA samples and related lab equipment listed at the beginning of the Technical Bulletin.
Poor results in downstream applications	RNase was introduced during the procedure	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. If more highly enriched mRNA is desired, adjust mRNA elution to 250µl with RNase-free water provided. Add 250 µl of 2 x Binding solution and continue with procedure at step 2 of the Technical Bulletin.
	Mini-prep capacity was exceeded	Re-purify as above. Next time use less RNA starting material.
Poor results in downstream applications	Salt carried over into eluate	Make sure beads are spun dry before adding Elution Solution.
	Improper storage or handling	Store eluted mRNA in elution buffer at -70°C or as ethanol precipitate at -70°C until needed. When mRNA solution is out of -70°C environment it should be placed on ice until needed.