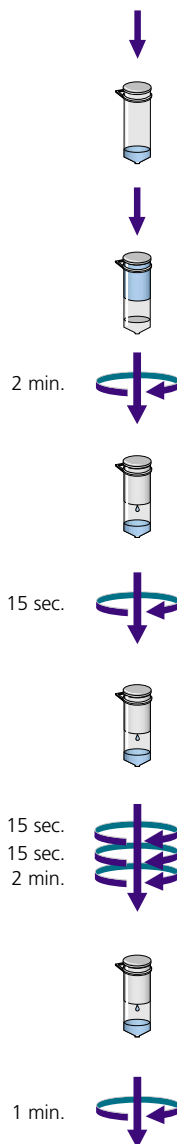


GENELUTE MAMMALIAN TOTAL RNA KIT

Cultured Cells or Tissue



Pure Total RNA

All spins at $\geq 14,000 \times g$

1 Release RNA from cells or tissues

- Add 2-mercaptoethanol to lysis solution (10 μ l 2-ME/1 ml lysis solution).
- Lyse cells/homogenize tissue in 250 or 500 μ l of lysis solution/2-ME mixture.
- Transfer lysate to blue filtration column. *Spin 2 minutes.*

2 Bind RNA to column

- Add equal volume of 70% ethanol to filtrate (250 or 500 μ l). *Mix thoroughly.*
- Transfer up to 700 μ l lysate/ethanol mixture to clear binding column. *Spin 15 seconds.*
- Discard flow-through & repeat if necessary.

3 Wash to remove contaminants

- Add 500 μ l wash solution 1 to column. *Spin 15 seconds.*
- Transfer column to new collection tube.
- Add 500 μ l wash solution 2 to column. **Note:** Ethanol must be added to wash 2 concentrate before first use. *Spin 15 seconds.* Discard wash solution.
- Add second 500 μ l wash solution 2 to column.
- Spin 2 minutes* to remove ethanol.

4 Elute purified RNA

- Transfer column to new collection tube.
- Add 50 μ l elution solution to column. *Spin 1 minute* (Repeat if $> 100 \mu$ g RNA expected).



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Problem	Reason	Solution
Binding column clogs	Sample size too large	For future preparations, use fewer cells or smaller tissue samples. To salvage the current preparation, spin longer than 15 seconds until solutions pass through the binding column. Yield of RNA will likely be reduced.
	Insufficient disruption	Vortex or pipet lysate until no cell clumps remain. Homogenize tissues in lysis solution until no visible particles remain.
Yield low or RNA degraded	Starting cells or tissues low in total RNA	Yields will vary greatly between different types of cells and tissues (see Expected Yield in technical bulletin).
	Old culture or tissue	Use cultures before they reach maximum density or become fully confluent, and harvest tissues as rapidly as possible.
	RNase-rich cells or tissue	Cells such as monocytes and macrophages, and tissues such as pancreas, spleen, and thymus, are rich in RNase and require immediate and thorough disruption in lysis solution to prevent degradation of RNA.
	Insufficient disruption	See above.
Downstream applications inhibited	Improper storage	To prevent RNase activity, tissues must be flash frozen in liquid nitrogen, stored at -70°C, and not allowed to thaw until they are disrupted in lysis solution.
	Residual Ethanol in eluate	Residual ethanol from wash solution 2 can inhibit enzymes, such as reverse transcriptase used for RT-PCR. After the final wash of the binding column, do not allow the wash solution to contact the column. Re-spin the tubes, if necessary.
	Residual Salt in eluate	Residual guanidine thiocyanate will also inhibit enzymes. Transfer the binding column to a clean receiving tube before adding wash solution 2. Wash twice with 500 µl of wash solution 2.