

Agilent Total RNA Isolation Protocol

Mammalian RNA Isolation

Short protocol for mammalian tissues and cells. Please consult manual (part number 5188-2710) for detailed instructions.

Homogenize tissue or cells

1. Prepare tissue or cell homogenate in appropriate amount of prepared lysis solution (10 μL β -ME/mL lysis solution).

Spin through mini-pretreatment column

2. Centrifuge up to 600 μL of homogenate through the mini-pretreatment column (natural), for 3 minutes at $16,000 \times g$.
3. Add an equal volume of 70% ethanol to the filtrate, mix and incubate 5 minutes.

Capture RNA on mini-isolation column

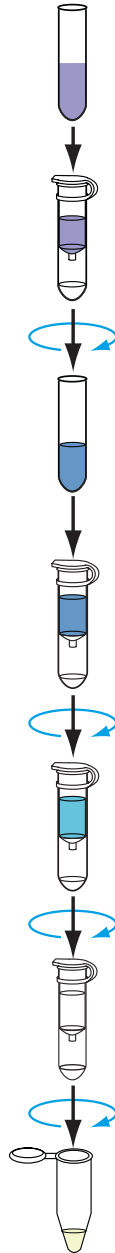
4. Add up to 600 μL of ethanol/lysis mixture to the mini-isolation column (blue), then centrifuge 30 seconds at $16,000 \times g$. Discard the flow-through, and replace the RNA-loaded column into the same collection tube.

Wash two times and spin dry

5. Add 500 μL of previously prepared wash solution (to which ethanol has been added) to the mini-isolation column, then centrifuge 30 seconds at $16,000 \times g$. Discard the flow-through, replace the mini-isolation column in the collection tube. Repeat step 5 one more time.
6. Spin the mini-isolation column for 2 minutes at $16,000 \times g$.

Elute pure intact RNA

7. Elute the purified RNA by addition of 10–50 μL of nuclease-free water, wait 1 minute, then centrifuge 1 minute at $16,000 \times g$.



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Bacterial RNA Isolation

Short protocol for bacteria. Please consult manual (part number 5188-2710) for detailed instructions.

Lyse sample

1. Resuspend pellet in 100 μL of lysozyme prepared in TE pH 8.0. Incubate to digest.
2. Add 200 μL of prepared lysis solution and mix.

Spin through mini-prefiltration column

3. Centrifuge homogenate through the mini-prefiltration column (natural), for 3 minutes at $16,000 \times g$.
4. Add 300 μL of 100% ethanol to the filtrate, mix and incubate 5 minutes.

Capture RNA on mini-isolation column

5. Add up to 600 μL of ethanol/lysis mixture to the mini-isolation column (blue), then centrifuge 30 seconds at $16,000 \times g$. Discard the flow-through, and replace the RNA-loaded column into the same collection tube.

Wash two times and spin dry

6. Add 500 μL of previously prepared wash solution (to which ethanol has been added) to the mini-isolation column, then centrifuge 30 seconds at $16,000 \times g$. Discard the flow-through, replace the mini-isolation column in the collection tube. Repeat step 5 one more time.
7. Spin the mini-isolation column for 2 minutes at $16,000 \times g$.

Elute pure intact RNA

8. Elute the purified RNA by addition of 10–50 μL of nuclease-free water, wait 1 minute, then centrifuge 1 minute at $16,000 \times g$.

