



Supplemental Protocol for DNA removal for RNA only recovery

Continue from Step 13 of the main protocol to perform DNase I digestion to remove DNA:

14. Allow magnetic beads to dry for 5 minutes at room temperature.

NOTE: Beads do not need to be completely dry, however, there should be no traces of liquid (i.e., droplets or puddles). If too much ethanol is left (more than 5 μ L), the DNase I activity will be inhibited, affecting DNA removal.

15. Remove the plate/tube from the magnet and add 100 μ L of DNase solution (prepared in Part A Step 4). Gently pipette mix 5 times while avoiding bubbles and foaming.

16. Cap the plate/tube. Incubate the samples in a water bath at 37°C for 15 minutes.

17. After incubation, directly add 200 μ L of Bind 2 Buffer into the reaction and pipette mix 10 times. Incubate the samples at room temperature for 5 minutes to allow rebinding.

NOTE: Bind2 is a clear solution and does not contain magnetic beads or require isopropanol.

18. Place the samples on the magnet for 5 minutes or wait for the solution to clear before proceeding to the next step.

19. Remove supernatant and discard. Wash by adding 800 μ L of 70% ethanol. Do Not Pipette Mix. Let sit for approximately one minute and then remove ethanol while samples remain situated on the magnet.

20. Repeat the above step one more time for a total of 2 ethanol washes. Completely remove residual ethanol.

21. Allow magnetic beads to dry for 10 minutes at room temperature. Beads do not need to be completely dry, but there should be no traces of liquid (i.e., droplets or puddles).

22. Remove the plate/tube from the magnet and elute RNA by adding a minimum of 20 μ L of nuclease free water. Pipette mix 10 times and incubate at room temperature for 2 minutes.

23. Return the plate/tube to the magnet for 2 minutes and carefully transfer eluted RNA into a fresh tube for storage and future analysis.

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If you have any technical questions, please feel free to contact [support@alinebiosciences.com](mailto:support@alinebiosciences.com) or 1-888-987-3677.

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