RiboGone - Mammalian (Cat. Nos. 634846 & 634847) allows for the specific removal of rRNA sequences (*5S*, *5.8S*, *18S*, and *28S*), as well as mitochondrial rRNA sequences (*12S*), from human, mouse, or rat total RNA. This kit is designed for use with limited sample amounts (10–100 ng of total RNA), and works with high- or low-quality RNA. Samples processed using the RiboGone kit are ready for cDNA synthesis with random primers.

I. Additional Materials Required

- Agencourt AMPure PCR Purification Kit (5 ml Beckman Coulter, Part No. A63880; 60 ml Beckman Coulter, Part No. A63881). This kit contains the magnetic SPRI beads to be used in this protocol.

  NOTE: Aliquot SPRI beads and allow them to come to room temperature for 30 min prior to use. Mix well to disperse before adding the beads to your reactions. The beads are viscous, so pipette them slowly.

- Magnetic separation device for 0.2 ml PCR tubes (see Appendix A)
- 80% ethanol

II. Protocol: rRNA Removal

The enzymes and buffers used in this protocol are supplied in the RiboGone kit.

1. Mix the following reaction components on ice. Perform each reaction in a separate 0.2 ml PCR tube.

Vol. Per Rxn	Reagent
1–4 µl	Total RNA sample (10–100 ng)
1 µl	5X RiboGone Hyb Buffer
0–3 µl	Nuclease-Free Water
5 µl	**Total volume**

2. Incubate the reactions in a thermal cycler at 95°C for 2 min; then ramp down the temperature (–0.1°C /sec) to 25°C.
3. Add 2 µl of RNAse H, 0.8 µl 10X RNaseH Buffer, and 1µl of RNAse Inhibitor to each reaction; mix well by vortexing and centrifuge briefly (~2,000 g) to collect the liquid at the bottom of each tube.
4. Incubate the reactions in a thermal cycler at 37°C for 30 min.
5. Add 4 µl of DNAse I to each reaction and incubate at 37°C for an additional 15 min.
6. Remove the reactions from the thermal cycler and add 7.2 µl of Nuclease-Free Water to each reaction for a total volume of 20 µl per reaction.
7. Add 36 µl of SPRI beads to each reaction and mix by pipetting the entire volume up and down 7–8 times.
8. Incubate the reactions at room temperature for 8 min.
9. Place the reaction tubes on a 96-well magnetic plate for 3–5 min or until all the reactions have become completely clear. Then, while the tubes are sitting on the magnetic stand, carefully pipette out the supernatants.
10. Add 200 µl of 80% freshly prepared ethanol to each sample without disturbing the bead pellets. Wait for 30 sec and carefully pipette out the supernatants.
11. Repeat Step 10 once, and then proceed to Step 12.
12. Centrifuge briefly (~2,000 g) to collect the residual ethanol at the bottom of the tubes. Place the tubes on the magnetic stand for approximately 30 sec, then remove all the remaining ethanol with a pipette.
13. Allow the tubes to stand at room temperature for 3–5 min until all the bead pellets appear dry.
14. Resuspend each bead pellet in 12 µl of RiboGone Purification Buffer by pipetting the entire volume up and down 7–8 times.
15. Return the tubes to the 96-well magnetic plate until they are completely clear. Then transfer 8 µl of each supernatant into a fresh tube. These are your final samples for use in cDNA synthesis reactions.

Appendix A: Constructing a Magnetic Separation Device for 0.2 ml PCR Tubes

It can be difficult to find magnetic separation devices designed specifically to handle 0.2 ml PCR strip tubes. Often, one can place strip tubes in a column/row of a magnetic separation device designed for use with 96-well plates. Alternatively, one can construct a suitable low-cost separation device from common laboratory materials.

Example 1: Using a 96-well separation device with strip tubes

As seen in Figure 1, you may place the tubes in the top part of an inverted P20 or P200 Rainin Tip Holder which is taped to a MagnaBot II Magnetic Separator (Promega Part No. V8351)

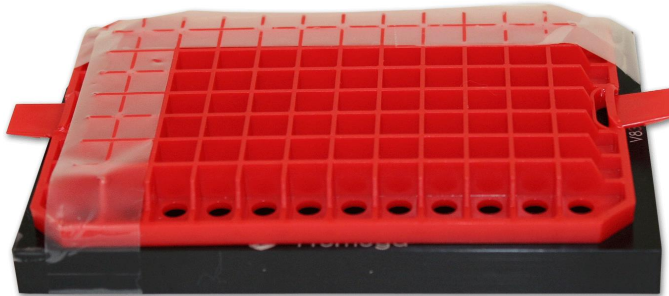

Figure 1. Setup for positioning 0.2 ml tubes containing first-strand cDNA on a MagnaBot II Magnetic Separator.

Example 2: Building a 0.2 ml tube magnetic separation device from rare earth bar magnets and a tip rack

As seen in Figure 2, neodymium bar magnets are taped together on the underside of the top section of a 20 µl tip rack (Panel A), and the rack is inverted so the tubes can be inserted (Panel B).

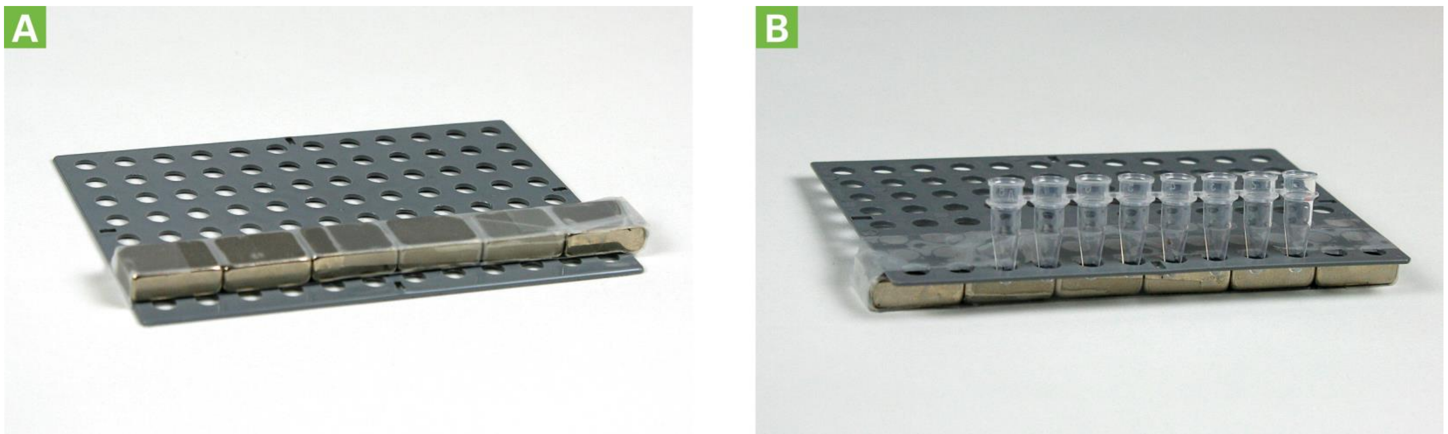

Figure 2. Constructing a magnetic separation device for 0.2 ml tubes from rare earth magnets. Panel A shows six 0.75" x 0.25" x 0.5" neodymium bar magnets (Applied Magnets Model # NB026) taped together on the underside of the top section of a 20 µl tip rack. Panel B shows the upright rack, into which an 8-tube strip of 0.2 ml tubes has been inserted

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This document has been reviewed and approved by the Clontech Quality Assurance Department.