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Seq-Star™ RNAClean and SmallEnrich Beads

Cat#: AS-MB-009

Instruction Manual version 1.0

Product Description

The Seq-Star™ RNAClean and SmallEnrich Beads system utilizes magnetic beads technology for rapid and reliable RNA purification after enzymatic reactions (*e.g.* DNase I treatment). The beads can also be used for selectively enrichment of large RNAs (>5S rRNA, ~120nt) and small RNAs (<5S rRNA) from total RNA samples (*Figure 1*).

Excess nucleotides, salts, enzymes and other residues can be efficiently eliminated by a simple binding and washing procedure. The high quality RNA products can be directly used in qPCR, microarray labeling and NGS library preparation, etc.

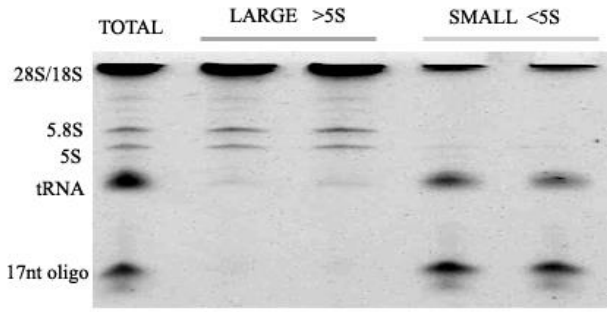


Figure1. The total RNA (>17 nt), large (>5S rRNA size) or small (<5S) RNAs are effectively partitioned and purified by Seq-Star™ RNAClean and SmallEnrich Beads, 7.5% urea-polyacrylamide gel electrophoresis showing. (7.5% urea-PAGE)

Kit components

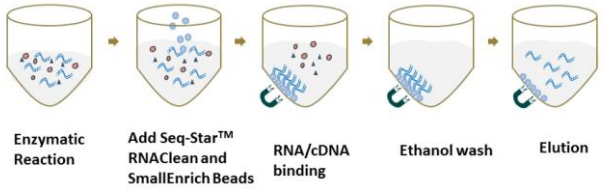
Cat#AS-MB-009	
Seq-Star™ RNAClean and SmallEnrich Beads	10mL
User Manual	√
Storage	4°C

Additional required materials

- Magnetic stand (tube compatible)
- Pipettors and pipette tips
- Vortexer
- 100% isopropanol
- Fresh 80% ethanol
- Elution buffer (RNase-free ddH_2O or TE buffer)

Protocols

RNA purification (>17nt)



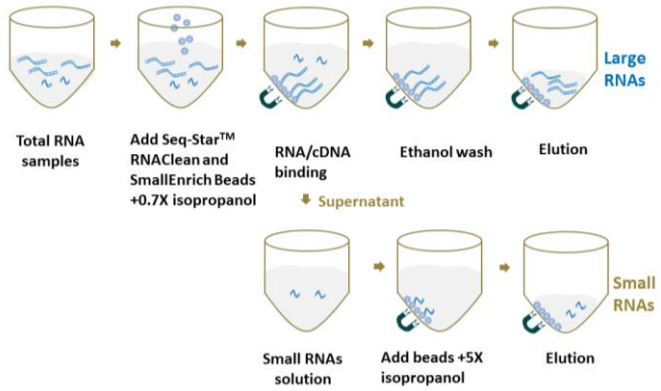
1. Gently shake the Seq-Star™ RNAClean and SmallEnrich Beads bottle to thoroughly resuspend the bead particles.
2. Add 1.8X (1.8:1 v:v ratio) resuspended beads and 4.2X 100% isopropanol to the RNA samples (*e.g.* 90 µL Beads and 210 µL isopropanol to 50 µL DNase I treatment reaction). Mix thoroughly by vortexing for 30 seconds.
3. Incubate the mix at room temperature for 10 minutes to allow the RNA to bind to the beads.
4. Place the mix tube on a magnetic stand until the supernatant becomes completely clear (about 3 minutes). Carefully aspirate and discard the supernatant.
5. Keep the tube on the magnetic stand and add 200 µL freshly prepared 80% ethanol (The volume of 80% ethanol should be larger than the original RNA and beads mix).
6. Incubate at room temperature for 30 seconds and then pipette off the supernatant.

Caution: Do not disturb the separated magnetic beads during your operation!

7. Repeat Step 6 once for a total of two washes. Make sure to remove all the ethanol from the bottom of the tube.
8. Air dry the beads at room temperature for 5 minutes.

   Note: Over-drying the beads may result in a lower recovery!
9. Take the tube off the magnetic stand and resuspend the beads in 10~20 µL elution buffer (RNase-free water or TE buffer) by pipetting up and down for 10 times.
10. Incubate at room temperature for 2 minutes.
11. Place the tube on the magnetic stand until the supernatant becomes completely clear from beads.
12. Transfer the supernatant containing the purified RNA to a new tube for downstream use.

Purification of small and large RNAs from total RNA into separate fractions



Large RNA Part

1. Gently shake the Seq-Star™ RNAClean and SmallEnrich Beads bottle to thoroughly resuspend bead particles.
2. Add 1.8X (1.8:1 v:v ratio) resuspended beads and 0.7X 100% isopropanol to the total RNA sample (*e.g.* add 90 µL bead suspension and 35 µL 100% isopropanol to 50 µL total RNA solution). Mix thoroughly by vortexing for 30 seconds.
3. Incubate the mix at room temperature for 10 minutes to allow large RNAs to bind to the beads.
4. Place the mix tube on a magnetic stand until the supernatant becomes completely clear (about 3 minutes). Transfer 175 µL of the supernatant to a new tube for small RNAs purification in **Small RNA Part** of the procedure.
5. Keep the tube on the magnetic stand and add 200 µL freshly prepared 80% ethanol to wash the beads.
6. Incubate at room temperature for 30 seconds and then pipette off the supernatant.

   Caution: Do not disturb the separated magnetic beads during your operation!
7. Repeat Step 6 once for a total of two washes. Make sure to remove all the ethanol from the bottom of the tube.
8. Air dry the beads at room temperature for 5 minutes.

   Note: Over-drying the beads may result in a lower recovery!
9. Take tube off the magnetic stand and resuspend the beads in 10~20 µL Elution buffer (RNase-free water or TE buffer) by pipetting up and down for 10 times.
10. Incubate at room temperate for 2 minutes.
11. Place the tube on the magnetic stand until the supernatant becomes completely clear from beads.
12. The supernatant contains the large RNAs fraction (>5S rRNA) from the total RNA.

Small RNAs Part

1. Add 1X resuspended RNAClean and SmallEnrich Beads and 5X 100% isopropanol to the supernatant from Step 4 in **Large RNAs Part**. For example, add 50 µL bead suspension and 250 µL isopropanol if the initial total RNA solution volume is 50 µL.
2. Mix thoroughly by vortexing for 30 seconds.
3. Incubate the mix at room temperature for 10 minutes to allow small RNAs to bind to beads.
4. Place the mix tube on a magnetic stand until the supernatant becomes completely clear (about 3 minutes). Carefully aspirate and discard the supernatant.
5. Keep the tube on the magnetic stand and add 400 µL freshly prepared 80% ethanol. The volume of 80% ethanol added should be larger than the original RNA and beads mix.
6. Incubate at room temperature for 30 seconds and then pipette off the supernatant.

   Caution: Do not disturb the separated magnetic beads during your operation!
7. Repeat Step 6 once for a total of two washes. Make sure to remove all the ethanol from the bottom of the tube.
8. Air dry the beads at room temperature for 5 minutes.

   Note: Over-drying the beads may result in a lower recovery!
9. Take tube off the magnetic stand and resuspend the beads in 10~20µL elution buffer (RNase-free water or TE buffer) by pipetting up and down for 10 times.
10. Incubate at room temperate for 2 minutes.
11. Place the tube on the magnetic stand until the supernatant becomes completely clear from beads.
12. The supernatant contains the small RNAs fraction (<5S rRNA) from the total RNA.

Troubleshooting

Problem	Possible cause	Suggestion
Low yield in the small RNA part procedure	Isopropanol concentration too low	Make sure the 100% isopropanol is well sealed during storage.
	80% ethanol is not fresh	Freshly prepare 80% ethanol before the purification
Problem in downstream applications using the purified RNA	Salt carryover	Completely aspirate off the supernatant after each RNA binding and wash step. Or carry out one more ethanol wash.