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Introduction

The Mag-Bind® RNA Cleanup Kit is designed for high-throughput purification of RNA or cDNA from in vitro applications such as transcription, antisense RNA amplification and cDNA probe synthesis using magnetic beads technology. This kit can also be used for cleanup of RNA that is purified with other total RNA isolation methods. This system combines Omega Bio-Tek's proprietary chemistries with the reversible nucleic acid-binding properties of paramagnetic beads to eliminate excess nucleotides, salts and other contaminants. The protocol can be used for both manual and fully automated processing. Purified RNA can be directly used most downstream applications.

Overview

The Mag-Bind® paramagnetic particles technology provides a better solution for nucleic acid purification than silica column based technologies. It can easily be scaled up and it provides very user-friendly handling procedures. If using the Mag-Bind® RNA Cleanup Kit for the first time, please read this booklet to become familiar with the procedures. RNA samples are first mixed with the paramagnetic particles with adjusted binding condition, to which RNA selectively binds. With just wash steps, trace contaminants such as nucleotides, salt and enzymes are removed. Pure RNA is eluted in RNase-free water and ready for downstream applications.

Storage and Stability

All components except Mag-Bind Particles solution in the Mag-Bind® RNA Cleanup Kit are stable for at least 12 months from date of purchase when stored at 22°C-25°C. The Mag-Bind Particles solution should be stored at 4°C.

Kit Contents

Product Number	M6248-00	M6248-01	M6248-02
Purification	5	50	200
Mag-Bind® Particles R	70 µl	550 µl	2.2 ml
VRB Binding Buffer	1 ml	5 ml	20 ml
LPA Buffer	55 µl	550 µl	2.2 ml
SPR Wash Buffer	6 ml	60ml	210ml
DEPC Water	1 ml	8 ml	25 ml
Instruction Manual	1	1	1

Before Starting

- Please read this entire booklet to become familiar with the Mag-Bind® RNA Cleanup Kit procedures.

- Dilute SPR Wash Buffer Concentrate with ethanol as follows and **store at room temperature**.

  M6248-00 Add14 ml absolute (96%-100%) ethanol.

  M6248-01 Add 140 ml absolute (96%-100%) ethanol to each bottle.

  M6248-02 Add 490 ml absolute (96%-100%) ethanol to each bottle.

- Dilute VRB Binding Buffer with ethanol (for regular RNA and cDNA cleanup) and isopropanol (for small fragment cleanup) as follows and **store at room temperature**:

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  M6248-00 Add 4 ml absolute ethanol (96%-100%)

  M1248-01 Add 20 ml absolute ethanol (96%-100%)

  M1248-02 Add 80 ml absolute ethanol (96%-100%)

- **Reaction Volume**: both the protocols in this user manual are based on 100µl reaction volume. If large than 100µl is to be used, please adjust the VRB binding buffer proportionally. There is not need to adjust the SPR Wash Buffer volume in the protocol.

Material to be Supplied by User

- 1.5 ml RNase-free centrifuge tube
- Absolute ethanol (96%-100%)
- Magnetic Separation Stand (i.e. Cat #MSTND-02)

Protocol for RNA and cDNA Cleanup

Note: This protocol is for RNA fragments larger than 200nt or DNA large than 150bp.

1. **Read the manufacturer's instruction manual for the magnetic separation stand, if provided**.

2. **Transfer the reactions into 1.5 ml RNase-free centrifuge tubes**.

3. **Add 10 µl Mag-Bind® Particles Solution to each sample**.

   Note: The Mag-Bind® particles tend to settle and bead together. The particles must be fully resuspended by shaking or vortexing before use.

4. **Add 2 volumes of VRB Binding Buffer diluted with ethanol to each sample.**

5. **Mix each sample by pipetting up and down 15 times or vortexing for 30 seconds, then incubate 5 minute at room temperature.** Mix again by pipetting up and down 4-5 times.

   Note: This step binds the RNA or cDNA to the Mag-Bind particles. For single stranded DNA, a 20 minutes incubation at room temperature is strongly recommended.

6. **Place the tube onto a magnetic separation stand suitable for micotube (Product# MSTND-02) for 5-10 minutes to magnetize the Mag-Bind® particles.** Use pipette tips to lightly stir the samples to ensure the complete capture of the particles on the sides of each well. Solution will clear when particles have completely migrated toward the magnets.

   Note: The seperation time will depend on the size of the reaction. Large volume requires more time for magnetic beads to separate. Wait until the solution is completely cleared before preceding next step.

7. **Slowly aspirate the cleared supernatant from the tube with pipet and discard the liquid.**

   Note: Do not disturb the beads pellet during liquid transfer.

8. **Take tube off from the magnetic separation stand. Add 1 ml of SPR Wash Buffer into each well of the plate and incubate 1 minutes at room temperature. Vortex the tube for 30 seconds to resuspend the beads.**

   Note: Do not disturb the beads pellet during liquid transfer.

9. **Place the tube back onto the magnetic stand. Wait 5 minutes to let the Mag-Bind particles to be cleared again. Aspirate the liquids from each well.**

   Note: Do not disturb the beads pellet during liquid transfer.

10. **Leave the tube on the magnetic stand. Add another 1 ml of SPR Wash into each well of the plate and incubate 30 seconds at room temperature. Aspirate the liquid out and discard.**

    Optional: **Wash the Mag-Bind particles again with another 1 ml SPR Buffer by repeating step 10.**

11. **Allow the tube to dry on the magnetic separation stand with cap open for 5-10 minutes.** Remove any liquid residue from the wells by pipetting.

    Note: Complete drying of samples is critical for removal of ethanol that might otherwise interfere with downstream applications. Avoid over-drying the samples, however, which will make resuspension and elution difficult in the following steps.

12. **After the samples have dried completely, remove the tube from the magnetic separation stand and add 40-60µl RNase-free water (supplied with DEPC-treated water) to each tube to elute the RNA from the Mag-Bind® particles.**

13. **Mix the Mag-Bind particles in each tube by pipetting up and down 10-20 times or vortexing for 30 seconds**. Incubate 2-3 minute at room temperature. Mix again by pipetting up and down 4-5 times.

    Note: In some situation, the Mag-Bind particles are spread around the wall of the tube after the mix. Centrifuge briefly to bring down the beads and incubate for 5 minutes. Also, if the beads are over dried, it is recommended to incubate the tubes overnight at 4°C for maximum recovery.

14. **Place the tube onto the magnetic separation stand to magnetize the Mag-Bind® particles.** Use pipette tips to lightly stir the samples to ensure the complete capture of the particles on the

sides of each well. Solution will clear when particles have completely migrated toward the magnets.

15. **Transfer the cleared supernatant containing purified RNA to a new RNase-free 1.5ml centrifuge tube**.

16. **Store the sample at 4° C if storage is only for a few days. For long-term storage samples should be kept at -20° C.**

Protocol for Cleanup Small fragments of RNA and cDNA

Note: This protocol is for RNA fragments small than 200nt or DNA less than 150bp. It can be also used for purify RNA from solution with lower RNA content.

1. **Transfer the reactions into 1.5 ml RNase-free centrifuge tubes.**

2. **Add 10 µl of LPA Buffer followed by 10 µl of Mag-Bind® Particles Solution to each sample.**

   Note: The Mag-Bind® particles tend to settle and bead together. The particles must be fully resuspended by shaking or vortexing before use.

3. **Add 4 volumes of VRB Binding Buffer diluted with ethanol to each sample**.

4. **Mix each sample by pipetting up and down 15 times or vortexing for 30 seconds, then incubate 5 minute at room temperature.** Mix again by pipetting up and down 4-5 times.

   Note: This step binds the RNA or cDNA to the Mag-Bind particles. For single stranded DNA, a 20 minutes incubation at room temperature is strongly recommended. Briefly centrifuge for 5 second to bring down the beads to the bottomof the tube if necessary.

5. **Place the tube onto a magnetic separation stand to magnetize the Mag-Bind® particles.** Use pipette tips to slightly stir the samples to ensure the complete capture of the particles on the sides of each well. Solution will clear when particles have completely migrated toward the magnets.

6. **Remove and discard the cleared supernatant.**

7. **Remove the tube containing the Mag-Bind® particles from the magnetic separation stand. Add 1 ml of SPR Wash Buffer diluted with ethanol to each well**.

8. **Mix each sample by pipetting up and down 4-5 times. Incubate 1 min at room temperature.** Mix again by pipetting up and down 4-5 times.

9. **Place the tube onto the magnetic separation stand to magnetize the Mag-Bind® particles. Mix with pipette tips to ensure the complete capture of the beads on the sides of each well.**

10. **Aspirate and discard the liquid from each well.**

11. **Leave the tube on the magnetic stand. Add another 1 ml of SPR Wash into each well of the plate and incubate 30 seconds at room temperature. Aspirate the liquid out and discard.**

12. **Allow the samples to dry on the magnetic separation stand for 5-10 minutes**. Remove any liquid residue from the wells by pipetting.

    Note: Complete drying of samples is critical for removal of ethanol that might otherwise interfere with downstream applications. Avoid over-drying the samples, however, which will make resuspension and elution difficult in the following steps.

13. **After the samples have dried completely, remove the tube from the magnetic separation stand and add 40-60µl RNase-free water (supplied with DEPC-treated water) to the tube to elute the RNA from the Mag-Bind® particles.**

14. **Mix each well by pipetting up and down 10-20 times. Incubate 2-3 minute at room temperature**. Mix again by pipetting up and down 4-5 times.

    Note: In some situation, the Mag-Bind particles are spread around the wall of the tube after the mix. Centrifuge briefly to bring down the beads and incubate for 5 minutes. Also, if the beads are over dried, it is recommended to incubate the tubes overnight at 4°C for maximum recovery..

15. **Place the tube onto the magnetic separation stand to magnetize the magnetic particles.** Mix with pipette tips to ensure the complete capture of the beads on the sides of each well.

16. **Transfer the cleared supernatant containing purified RNA to a new Rnase-free tube and store at -20°C**.

Centrifugal Protocol

Note: Please read through previous sections of this manual before using this protocol.

1. **Prepare samples by following the standard protocol in previous sections.**

2. For all binding, washing and elution steps. Instead to use the magnetic separation device to collect the Mag-Bind particles, centrifuge the tube or plate at 14,000 x g (for tube)for 1 minute or 3000 x g for 3 minutes to collect the magnetic beads.

Troubleshooting

Problem	Cause	Suggestions
Low yield	Low PCR product yield	Increase the reaction volume and repeat the purification.
	Smaller RNA product size	Small RNA or cDNA fragments normally give lower yield. See page 6 for optimized protocol very small fragments
	Ethanol residue	During the drying step, remove any liquid from bottom of the well
	Particle loss during the procedure	Increase magnetization time. Aspirate more slowly
	RNA remains bound to beads	Increase elution volume to 100 µl and incubate for 20 minutes at room temperature.
	Incompletely resuspension of the beads during elution	Fully suspend the beads by pipetting up and down.
	Mag-Bind Particles are over dried.	After resuspend the beads with water, incubate the plate at 4°C for overnight
Non-specific RNA products were not removed	The size of the non-specific RNA or cDNA products are larger than 100bp.	Non-specific RNA or cDNA products larger than 200nt will not efficiently removed
Problems in downstream applications	Salt carry-over.	SPR Wash Buffer must be stored at room temperature.
	Ethanol carry-over	Ensure the beads are completely dried before elution