RNA MagClean™ DX RNA Cleanup

Catalog Numbers: **C-1005-5, C-1005-50, C-1005-200**

Contents

Protocol Manual *Revision 2.1*

Please refer to http://www.alinebiosciences.com Support section for updated protocols and MSDS when handling or shipping any chemical hazards. The information provided is subject to change without notice.

INTRODUCTION

The RNA MAGCLEAN™ DX purification system utilizes a proprietary magnetic bead based technology for quick and efficient, high-throughput purification of RNA from RT and other enzyme reactions. RNA

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MAGCLEAN™ DX utilizes an optimized buffer for selective binding of RNA to magnetic beads. The protocol mainly consists of binding, washing and elution steps. Primers, nucleotides, salts and enzymes in reaction mixture are removed during the binding and washing steps. The purified RNA product is essentially free of contaminants and suitable for reverse transcription or Next Gen sequencing library construction.

RNA MAGCLEAN™ DX can be used in the following applications:

- ✓ Reverse transcription
- ✓ RT-PCR
- ✓ Genotyping and SNP detection
- ✓ Next gen library construction
- ✓ cRNA Cloning

The RNA MAGCLEAN™ DX is adaptable to various commercially available automation platforms.

PROCESS OVERVIEW

1. Add 18ul RNA MagClean™ DX per 10ul of RNA sample for RNA binding to magnetic beads.

2. Separate beads and RNA from unbound contaminants.

3. Wash beads twice with 80% Ethanol to remove salt and contaminants (Fresh 70% ethanol may be used instead).

4. Elute purified RNA from magnetic beads to a new plate.

SPECIFICATIONS

The RNA MagClean™ DX kit can be performed in a tube, 96 well and 384 well formats. The following table illustrates the number of clean up reactions RNA MagClean™ DX kits can perform depending on the sample volume of RNA sample.

RNA MAGCLEAN™ KITS

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RNA MAGCLEAN™	P/N
RNA MAGCLEAN™ DX - 5 mL	C-1005-5
RNA MAGCLEAN™ DX - 50 mL	C-1005-50
RNA MAGCLEAN™ DX - 200 mL	C-1005-200

Number of reactions:

RNA Sample Volume (uL)	C-1005-5 (# of reactions)	C-1005-50 (# of reactions)	C-1005-250 (# of reactions)
10	277	2777	11111
20	138	1388	5555
50	55	555	2222

MATERIALS

Supplied in the Kit:

RNA MagClean™ DX magnetic bead Solution

- **Store at 4°C upon arrival (DO NOT FREEZE),** for up to 12 months
- **Mix the reagent well at room temperature** to completely resuspend beads prior to use. It should appear visually homogenous.

To be supplied by the User:

Apparatus

Name	Recommended Model	Recommended Vendor and P/N
96-well PCR reaction plate	96-well round-bottom microtiter plate	Corning, Inc., # 3797, www.corning.com Fisher Scientific # 07-200-105, www.fishersci.com
	96 well cycling plate	ABgene Limited, # AB-0800, AB-1000, AB-1400, www.abgene.com worldwide and Fisher Scientific www.fishersci.com in the U.S.
Magnetic PCR plate	96 well ring stand	Ambion Inc., (acquired by Applied Biosystems), # AM10050, www.appliedbiosystems.com

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PCR Plate Seals	Easy Peel Heat Sealing Foil	ABgene Limited, # AB-3739 and AB-3739/s, www.abgene.com worldwide and Fisher Scientific www.fishersci.com in the U.S.
Liquid handling robotics		
Multichannel hand pipette		

Reagents

Reagents	Application
80% ethanol (or freshly made 70% ethanol)	Washing
10 mM TRIS-HCl, pH8.0	RNA elution
Reagent grade water	

PROCEDURE - 96 WELL FORMAT

1. **Determine whether or not it is necessary for a plate transfer.**

   Note: If the RNA sample volume multiplied by 2.8 exceeds the volume of the 96 well plate, a sample transfer to a 300 uL round bottom plate is required.

2. **Shake gently the RNA MagClean™ DX bottle to resuspend any magnetic particles that may have settled. Add RNA MagClean™ DX according to the table below:**

RNA sample Volume (µL)	RNA MagClean™ Volume at 1.8X (µL)
10	18
20	36
50	90

Note: Use the following equation to determine the volume of RNA MagClean™ DX:

(Volume of **RNA MagClean™** DX per reaction) = 1.8 x (RNA sample Volume)

3. **Mix RNA MagClean™ DX and RNA sample thoroughly by pipette mixing 5 times.**

   Note: After mixing, the color of the mixture should appear homogenous.

4. **Incubate the mixed samples for 5 minutes at room temperature for maximum recovery.**

5. **Place the reaction plate onto a 96 well magnet plate for 3 minutes or wait until the solution is clear.**

   Note: Wait until the solution is clear before proceeding to the next step. Otherwise unsettled beads may become lost.

6. **Aspirate the cleared solution from the reaction plate and discard.**

   Note: This step must be performed while the reaction plate is placed on the magnet plate. Avoid disturbing the settled magnetic beads. If beads are drawn into tips, eject for the beads to resettle and leave behind a few microliters of solution.

7. **Dispense 200 µL of 80% ethanol to each well of the reaction plate and incubate for 30 seconds at room temperature. Aspirate the ethanol and discard. Repeat for a total of two washes.**

   Note: It is important to perform these steps with the reaction plate on a magnetic plate. Do not disturb the settled magnetic beads. Remove all of the ethanol from the bottom of the well to avoid ethanol carryover.

   A 5 min air dry at room temperature is recommended for the evaporation of the remaining traces of ethanol. Do not over-dry the beads (bead ring appears cracked) as this will significantly decrease elution efficiency.

8. **Take off the plate from the magnetic plate, add 40 µL of elution buffer (Reagent grade water, TrisHCl pH 8.0) to each well of the reaction plate and pipette mix 5 times.**

   Note: More than 40 µL of elution buffer can be used, but using less than 40 µL will require extra mixing to ensure the liquid comes into contact with the beads and may not be sufficient to elute the entire PCR product. Elution is quite rapid and it is not necessary for the beads to go back into solution for it to occur.

9. **Place the reaction plate onto a 96 well Magnetic Plate for 1 minute to separate beads from the solution.**

10. **Transfer the eluate to a fresh plate for storage and analysis.**

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