Solid Phase Reverse Immobilization (SPRI) Bead Technology for Micro RNA Clean Up using the Agencourt RNAClean XP Kit

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Introduction

Micro RNAs are small non-coding ribonucleic acids with sizes between 18 and 40 nucleotides (nts). The majority of miRNAs are composed of approximately 22 nts. Purification of these small miRNA fragments after enzymatic reactions can be challenging because most commonly-used column methods were designed to recover fragment sizes that are greater than 50 nts. Therefore, these column methods often produce lower miRNA yields.

This application note describes the use of Beckman Coulter's Agencourt RNAClean XP kit for miRNA clean up and concentration of miRNA and total RNA from low yield samples. The RNAClean XP kit is a Solid Phase Reverse Immobilization (SPRI) paramagnetic bead-based method for RNA and cDNA purification. The magnetic bead procedure does not require centrifugation or filtration steps. It is a simple, rapid, automation-friendly protocol that produces high quality samples.

miRNA purification is achieved in three steps.

Step 1: Mix miRNA with binding buffer containing beads and isopropanol.

Step 2: Wash the miRNA bound beads with fresh 85% ethanol.

Step 3: Elute the miRNA from the beads with nuclease free water.

The results show that the SPRI bead purification protocol gives two times higher miRNA recovery compared to a column purification method.

Materials and Methods

Different amounts of the RNAClean XP reagent, 100% isopropanol and input sample volume were tested to achieve optimal binding buffer conditions for miRNA purification. Table 1 shows the ratio of the binding buffer to sample volume for miRNA purification. For example, 50 µL of sample containing 1 µg RNA (Life Technologies, AM7950) was mixed thoroughly with 90 µL of RNAClean XP (Beckman Coulter, A63987) plus 270 µL of 100% isopropanol (American Bioanalytical, AB07015) in a 96 well plate format (ABGene, T5050G), and incubated at room temperature for 5 minutes. The sample plate was placed on an Agencourt SPRIPlate 96-Ring Super Magnet Plate (Beckman Coulter, A32782) for 15 minutes or until the solution turns completely clear to separate the magnetic beads. The RNA bound beads were washed two times with 300 µL of freshly-made 85% ethanol, prepared from 100% ethanol (American Bioanalytical, AB000138). The beads were air dried for 5 minutes and RNA was eluted with 50 µL of nuclease free water.

For miRNA clean up and purification using the MinElute Column modified protocol (Qiagen, 74204): 100 µL of sample containing 1 µg RNA was mixed thoroughly with 350 µL RLT +250 µL 100% ethanol. The sample mixture was applied to the first column and centrifuged for 15 sec at 10,000 rpm. 50 µL of RLT and 500 µL 100% ethanol were then added into the collected flow through. The flow through was mixed well with RLT+100% ethanol and was transferred to the second column. After the second centrifugation for 15 sec at 10,000 rpm, the column was washed with RB buffer and then with 100% ethanol, the purified miRNA was eluted with 50 µL of nuclease free water.

The concentration and purity of the miRNA and RNA purified from both RNAClean XP and MinElute Column was measured with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). 1 µL of. purified miRNA was analyzed by an Agilent Small RNA Kit (Agilent Technologies, 5067-1548) using the 2100 Bioanalyzer (Agilent Technologies).

MicroRNA let-7c gene expression was determined by Taqman microRNA assay (Life Technologies 4427975, assay ID000379). 50 ng and 100 ng of total RNA was used for the reverse transcription reaction using the TaqMan micro RNA Reverse Transcription kit (Life Technologies, 4366596) and 1.33 µL of cDNA was used per PCR reaction in triplicate using Taqman Universal Master Mix II (Life Technologies, 4440038). For the RT and qPCR reaction set up, see details in reference IB-17265A (www.Beckman.com).

AAG-245APP06.14-A

SAMPLE VOLUME (µL)	RNAClean XP (µL)	100% ISOPROPANOL (µL)
50	90	270
100	180	270
150	270	270
200	360	270

Table 1: Binding buffer formulation for miRNA clean up

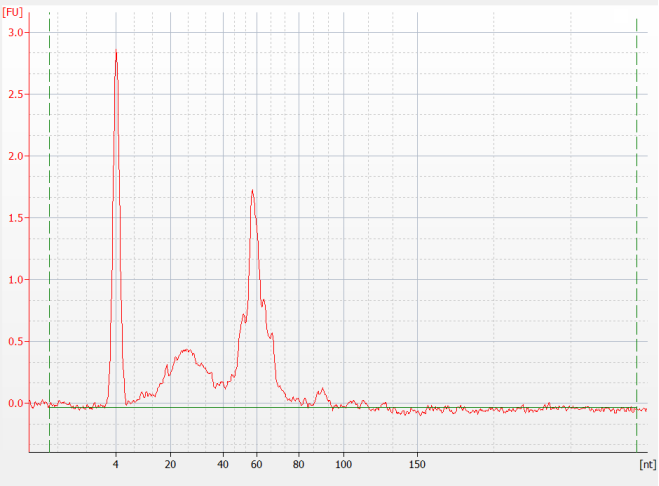
Results and Discussion

RNAClean XP resulted in highly efficient miRNA and small RNA recovery.

A 50 µL RNA sample containing miRNAs with sizes between 18-60 nts was purified using the RNAClean XP reagent and eluted with 50 µL of nuclease free water. 1 µL of the pre-purified control miRNA and post-purified, eluted miRNA were analyzed on the Agilent Small RNA Chip to determine miRNA recovery. Figure 1 shows that the estimated recovered miRNA amount was not significantly different from pre- and post-purified samples. Figure 1 shows an example of the miRNA profiling. The estimated miRNA concentration of pre-purified control sample was at 554 pg/µL with 67% miRNA (left panel), and the estimated purified miRNA concentration was at 541 pg/µL with 63% miRNA (right panel). The average estimated miRNA concentration purified from a total of four samples was at 663 pg/µL+/-93 with 62%+/-2.9 of miRNA (data not shown).

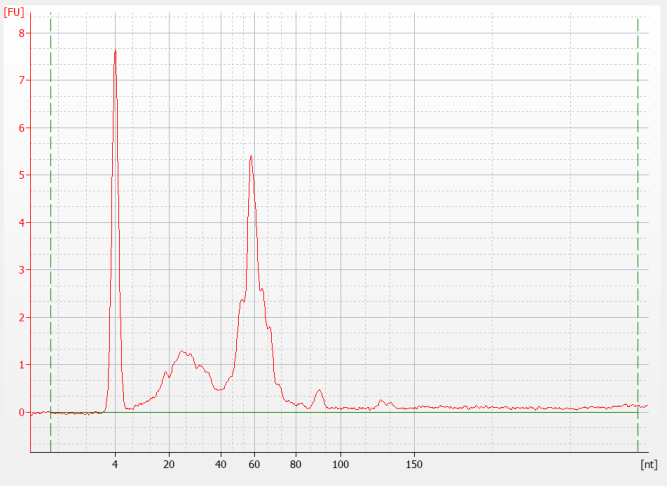
Figure 1. RNAClean XP gave very high miRNA recovery

Pre-purified Sample



Small RNA Concentration (pg/µL)	828.1
miRNA Concentration (pg/µL)	554.2
miRNA / Small RNA Ratio (%)	67

Post-purified Sample



Small RNA Concentration (pg/µL)	862.0
miRNA Concentration (pg/µL)	541.4
miRNA / Small RNA Ratio (%)	63

RNAClean XP protocol showed higher miRNA recovery than the column method.

100 µL of RNA containing 20 ng/µL of total RNA plus miRNA (Life Technologies, AM7950) was used to determine miRNA plus RNA recovery. Eight samples were purified using the RNAClean XP protocol (Beckman Coulter, AAG-244APP06.14-A) and eight samples were purified using the MinElute Column method. The results showed that the RNAClean XP method gave higher miRNA plus RNA yields compared to the column method (Table 2). In an independent experiment, 100 µL of samples containing 5.5 ng/µL of total RNA plus miRNA (Life Technologies, AM7950) were tested for RNA recovery, eight samples were purified using each method. The data consistently showed that RNAClean XP gave 80 - 94% recovery, whereas the column method only had 39 - 49% recovery (Table 3). It is important to note that majority of the larger RNA fragments were removed in the column method.

METHOD	AVERAGE RNA RECOVERY +/- STD DEV	AVERAGE % RECOVERY
RNAClean XP	16 ng/µL +/- 1.34	80%
MinElute Column	7.76 ng/µL +/- 3.16	39%

Table 2: Average RNA and miRNA recovery from RNAClean XP and MinElute column.

METHOD	AVERAGE RNA RECOVERY +/- STD DEV	AVERAGE % RECOVERY
RNAClean XP	5.2 ng/µL +/- 0.55	94%
MinElute Column	2.7 ng/µL +/- 0.64	49%

Table 3: Average RNA and miRNA recovery from RNAClean XP and MinElute column.

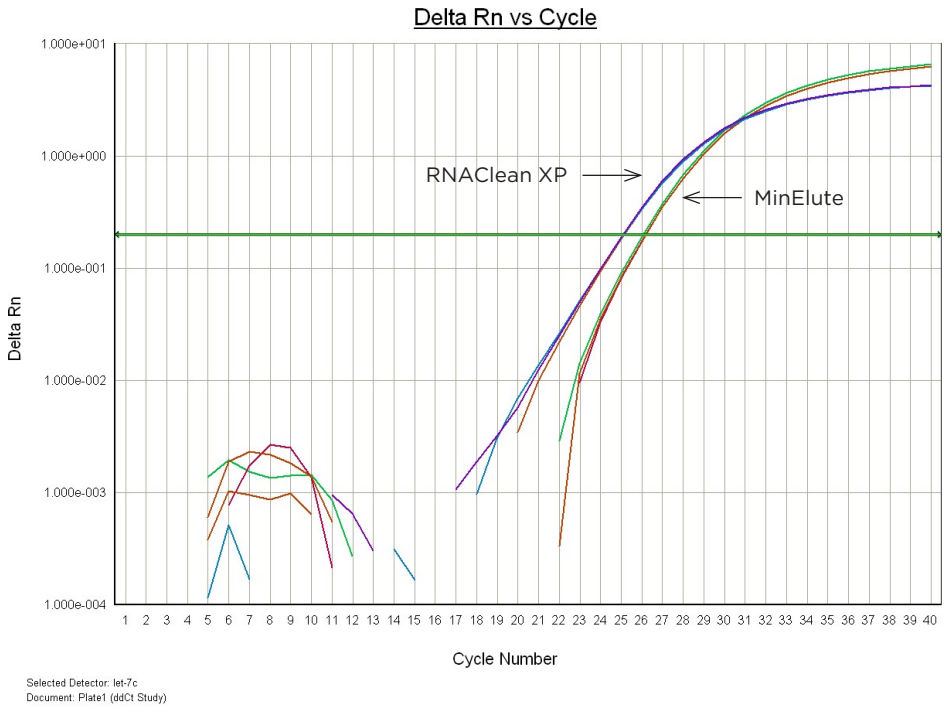
RNAClean XP purified samples showed higher miRNA let7c gene expression.

To determine the miRNA recovery efficiency, 50 ng of the purified RNA samples from the RNA Clean XP and MinElute column were used to determine miRNA let-7c gene expression using the Taqman microRNA assay (Life Technologies 4427975, assay ID000379). The results show that the average cycle threshold (Ct) for RNAClean XP purified RNA samples was 25 cycles, whereas the MinEluted purified RNA samples gave an average Ct at 26 cycles in 50 ng per reaction (Table 4 and Figure 2), suggeting that RNAClean XP has higher let 7c gene expression as compared to the column method. In consistent with this finding, 2 times more miRNA input amount was needed from the MinElute purified sample in order to archieve the same cycle threshold for let 7c gene expression (data not shown).

METHOD	AVERAGE RNA RECOVERY +/- STD DEV
RNAClean XP	24.98 +/- 0.145
MinElute Column	26.30 +/- 0.03

Table 4: Average Ct value for the let-7c gene expression in a 50 ng reaction, a total of three independent samples were tested for each method.

Figure 2. Overlaid amplification plots from RNACleanXP and MinEluted purified RNA samples.



Conclusion

The data from this study shows that RNAClean XP has higher miRNA recovery when compared to the column miRNA purification method. The RNAClean XP protocol enables recovery of both miRNA and total RNA in one procedure. It does not require centrifugation, filtration or precipitation steps. It provides scalable throughput as well as being automation-friendly.

Reference

For miRNA size selection and enrichment, use the SPRIselect reagent (Beckman Coulter, B23318) and the miRNA enrichment supplemental protocol (IB-18479A, www. Beckman.com).