

# MicroRNA and RNA Extraction from plasma and serum using Beckman Coulter's Agencourt RNAdvance Blood Kits

## APPLICATION NOTE



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MicroRNAs (miRNAs) are small non-coding ribonucleic acids (~22 nt) that play important roles in gene regulation by binding to and repressing the activity of specific target mRNAs. It is known that tumor cells release miRNAs and RNA into the blood circulation. Profiles of miRNAs and RNA in plasma and serum have been found to be altered by cancer and other disease states. Therefore, discovery of cell-free circulating miRNAs and RNA biomarkers in the bloodstream or body fluids provides for the possibility of non-invasive diagnosis in early stages.

This application note describes the purification of miRNA and RNA from plasma and serum using Beckman Coulter's Agencourt RNAdvance Blood kit. Beckman Coulter's SPRI (Solid Phase Reverse Immobilization) paramagnetic bead-based chemistry provides an easy, rapid, high yielding, robust and automation-friendly nucleic acid purification procedure that does not require vortexing, centrifugation or filtration steps. The data shows that same protocol can be used for miRNA and RNA extraction from plasma and serum. The spiked in miR39 control recovery is comparable to a commonly used column kit.

### Materials and Methods

200 $\mu$ L of frozen bovine plasma K2-EDTA, mouse plasma K2-EDTA and mouse serum (LAMPIRE Biological Laboratories, 7300807, 7304307, and 7324300) or human plasma K2-EDTA was digested with 300 $\mu$ L lysis buffer and 20 $\mu$ L of proteinase K for 15 min at 55°C. RNA was extracted using an RNAdvance Blood kit (Beckman Coulter, A35605). Human whole blood was collected in a K2-EDTA anticoagulant tube (Avena Medica) from consenting healthy adults. Plasma was separated by centrifugation of 1500 RCF for 10 min at 4 degrees using Beckman Coulter Allegra X-22R and rotor SX4250 (392187 and 392243). The plasma supernatant was transferred and

pooled into a nuclease free clean tube, and used for miRNA extraction. A control non-human synthetic cel-miR39 (Qiagen, 219610) was spiked into the bovine plasma to determine the optimal binding and washing conditions for miRNA recovery using the RNAdvance Blood plasma miRNA supplemental protocol (Beckman Coulter, AAG-1021SP07.15-A). Samples were eluted in 40 $\mu$ L of nuclease free water in the final elution step. Control miR39 recovery was measured using a quantitative Taqman qPCR assay (Life Technologies, 4427975, assay ID 000200). miRNA (let7c, miR16, miR21, miR129 and miR155) gene expression was determined using a Taqman microRNA assay (Life Technologies, 4427975 assays ID000379, 000391, 000397, 002298 and 002623 respectively). 5 $\mu$ L of eluted RNA was used for the reverse transcription reaction using the TaqMan micro RNA Reverse Transcription Kit (Life Technologies, 4366596) and 1 $\mu$ L of cDNA was used per 10  $\mu$ L PCR reaction in triplicate, using Taqman Universal Master Mix II (Life Technologies, 4440038). For messenger RNA gene expression, 1 $\mu$ L of eluted RNA was used for cDNA synthesis using a random primer (Life Technologies, 4368814), and 1 $\mu$ L of the cDNA was used for a 10 $\mu$ L PCR reaction using prime time qPCR assays (Integrated DNA Technologies). The primer probe assay ID's used for the ACTB, B2M, GAPDH and HPRT1 were Hs.PT.39a.22214847, Hs.PT.39a.22214845, Hs.PT.39a.22214836 and Hs.PT.39a.22214821 respectively).

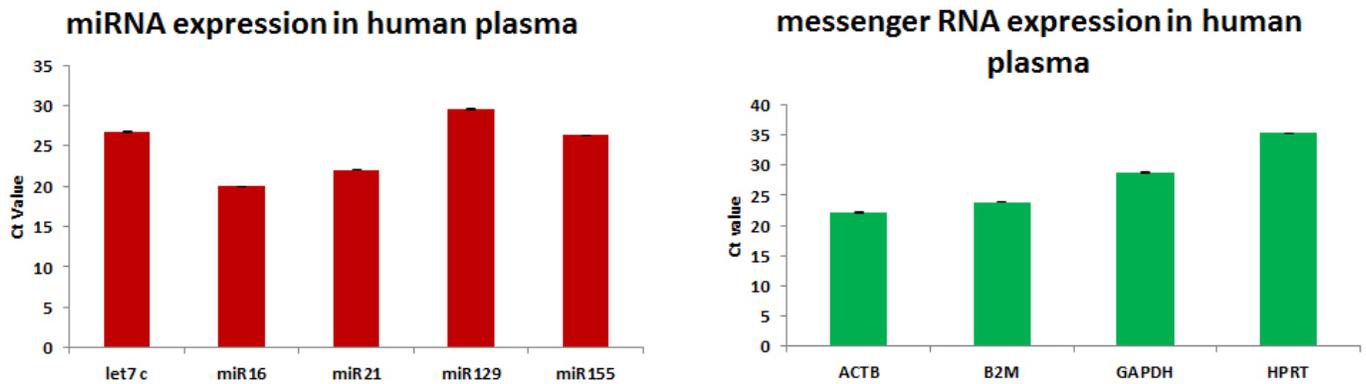


Figure 1. miRNA gene expression (left) and messenger gene expression (right) from 200 $\mu$ L of human plasma.

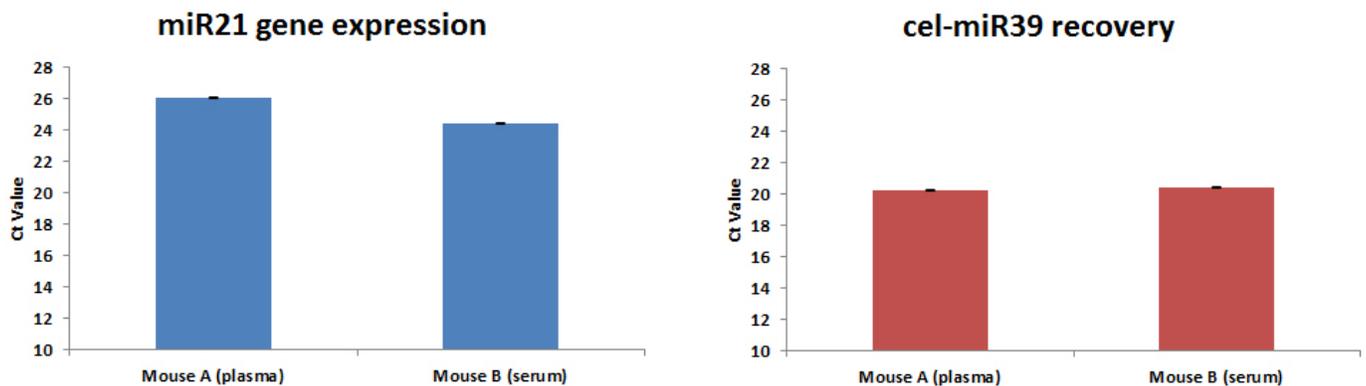


Figure 2. miR21 gene expression (left) and control miR39 gene expression (right) from 200 $\mu$ L of from mouse plasma and serum.

## Results and Discussion

MiRNA and messenger RNA gene expression data demonstrates that circulating miRNA and RNA were successfully extracted from human plasma samples.

5 $\mu$ L of eluted RNA was used for let 7c, miR16, miR21, miR129, and miR155 gene expression. The average cycle threshold (Ct) was calculated from duplicate samples. The average Ct values for let 7c, miR16, miR21, miR129 and miR155 target gene expression human plasma were 26.70 $\pm$ 0.08, 19.94 $\pm$ 0.03, 22.03 $\pm$ 0.02, 29.52 $\pm$ 0.05 and 26.33 $\pm$ 0.03 respectively (Figure 1, left). This result indicates that miRNA was successfully extracted from plasma utilizing the RNAAdvance Blood kit. The data showed that miR16 and miR21 were the most highly-expressed genes and miR129 was lowest expressed gene in circulating blood. For messenger RNA gene expression, ACTB, B2M, GAPDH and HPRT1 genes were used for evaluation. The average Ct values for ACTB, B2M, GAPDH and HPRT1 gene expression were 22.11 $\pm$ 0.06, 23.88 $\pm$ 0.03, 28.73 $\pm$ 0.07 and 35 $\pm$ 0.06 respectively (Figure 1 Right). ACTB was the most highly expressed gene and HPRT was the lowest expressed gene in circulating blood. The controls with no template showed no amplification, indicating that the amplification resulted from miRNA or messenger RNA alone (data not shown).

## RNAAdvance Blood kits can be used for serum and plasma samples

To determine whether the plasma miRNA extraction protocol can be used for serum, two replicates of frozen plasma from mouse A and frozen serum from mouse B were spiked in the miR39 control as an internal control. The results showed that both plasma and serum gave comparable miR39 recovery. The average cycle threshold (Ct) value of the spiked miR39 in the serum was 20.39 $\pm$ 0.063 and 20.20 $\pm$ 0.044 in plasma (Figure 2, right). For miR21 gene expression, there was a two cycle difference between these mice. The average cycle threshold (Ct) value for miR21 was 26.45 $\pm$ 0.019 in the plasma and 24.64 $\pm$ 0.059 in the serum (Figure 2, left). Figure 3 shows examples of the overlaid amplification curves for miR21 (left) and miR39 control (right).

## The RNAAdvance Blood kit gave comparable control cel-miR39 recovery when compared to a column kit.

Two replicates of human plasma were spiked in cel-miR39 control and the miR39 recovery was measured by miRNA qPCR assay. The average cycle threshold (Ct) value for spiked in control miR39 expression in human plasma was 20.09 $\pm$ 0.053 using RNAAdvance Blood kit and 20.54 $\pm$ 0.039 using miRNeasy serum/plasma kit (Qiagen, 217184). The

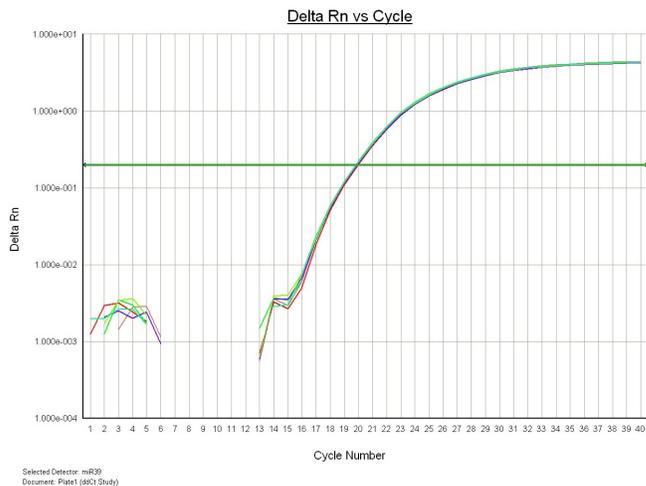


Figure 3. Overlaid miR21 (left) and miR39 (right) amplification plots.

results indicate that both kits gave comparable miR39 recovery efficiency (Figure 4).

### Conclusions

The data from this study shows that Beckman's RNAAdvance Blood Kit can be used for miRNA and RNA extraction from plasma and serum. The magnetic bead-based extraction protocol provides scalable throughput and it is automation-friendly.

### Acknowledgements

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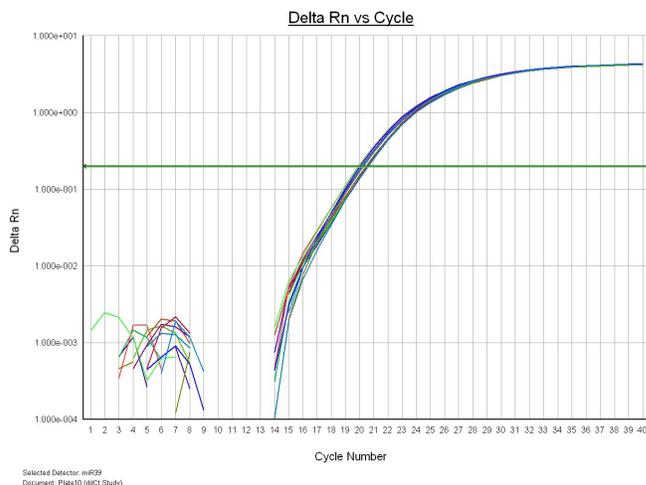


Figure 4. Overlaid amplification plots of miR39 from two replicates of human plasma extracted from column and RNAAdvance Blood kits.

