MicroRNA Extraction from Exosomes using Beckman's Agencourt RNAdvance Cell v2 Kits

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Summary

Exosomes are cell-derived vesicles that are present in different types of biological fluids, such as blood, urine, and cultured medium of cell cultures. Exosomes are typically defined as those nano-sized vesicles (bewteen ~30 nm-150 nm diameter) released from the cell when multi-vesicular bodies fuse with the plasma membrane, although some similarly sized vesicles may be released directly from the plasma membrane and co-purified along with exosomes. These circulating extracellular vesicles carry a variety of functional components including DNA, proteins, lipids, RNA, non-coding RNA and microRNA. The delivery of miRNA and other molecules can function as intercellular communication vehicles by transferring these signaling molecules between specific cells. Therefore, exosomes have the capacity to influence a larger diversity of genes' expressions and regulate biological pathways such as tumorigenesis and immune response. Studying exosome gene expression profiling becomes important and could shed light on the cancer-associated RNA biomarkers for cancer diagnostics and therapeutics.

This application note describes the purification of miRNA and RNA from exosome samples isolated from cell culture medium, using Beckman's Agencourt RNAdvance Cell v2 kit. The method uses Beckman Coulter's SPRI (Solid Phase Reverse Immobilization) paramagnetic bead-based chemistry, which 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 miRNA and coding RNA was successfully extracted from exosomes.

Materials and Methods

Exosomes from IMR-90 and Jurkat cells were isolated via differential ultracentrifugation procedure. Cells were grown to at least 10% confluency before adding exosome collection media (EMEM for IMR-90, RPMI 1640 for Jurkat cells) containing 10% FBS. Endogenous bovine exosomes from the FBS were pelleted and removed prior to cell incubation by ultracentrifugation at 100,000 x g for 18 hours (Beckman Coulter Optima L-80 XP Preparative Ultracentrifuge, SW 32 Ti rotor). Cells were incubated for about 24-48 hrs until the cell growth reached a plateau. The cultured media was removed and subjected to a series of centrifugation steps (Sorvall Legend X1R centrifuge, Fiberlite F15-8x50cy fixed-angle rotor) as described: low speed spins at 300 x g for 10 min, 2000 x g for10 min, and 10,000 x g for 30 min. The supernatant from each step of centrifugation was retained and the pellet was discarded to remove cells, dead cells, and cell debris respectively. Cleared supernatant was concentrated by a factor of 10 and ultracentrifuged at 100,000 x g for 70 mins (Beckman Coulter Optima L-80 XP Preparative Ultracentrifuge, SW 32 Ti rotor). Pellet was re-suspended in ~24mL of fresh 1X PBS to re-fill the centrifuge tube and the 100,000 x g, 70 min ultracentrifugation step was repeated once more to remove co-precipitated free protein, such as BSA. This final exosome-containing pellet was re-suspended in ~100 µL of fresh 1 X PBS and a small aliquot (1µL) was diluted x5 and lysed (RIPA Lysis Buffer, Upstate® Cat.#20-188) for protein

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concentration measurement by BCA assay (Pierce® BCA Protein Assay Kit, Cat.#23225). A second aliquot was diluted 1500x in freshly filtered (via 0.1 µm Nylon syringe filter) PBS and exosome size distribution and number concentration measured by Nanoparticle Tracking Analysis (NTA, NanoSight LM10) at room temperature. 10µL of the frozen purified exosome (protein concentration 1.5mg/mL) was digested with 60 µL of lysis buffer and 3µL of proteinase K for 30 min at room temperature and RNA was extracted using an RNAdvance Cell v2 kit (Beckman Coulter, A47942). Two samples were used for miRNA extraction using the RNAdvance Cell v2 miRNA supplemental protocol without DNase treatment (Beckman Coulter, AAG-850SP03.15-A). Samples were eluted in 25µL of nuclease free water in the final elution step. 1µL of RNA was analyzed using an Agilent RNA 6000 Pico chip (Agilent Technologies, 5067-1513) on the 2100 Bioanalyzer (Agilent Technologies). miRNA (let7c, miR16, miR21, miR155 and RNU44) gene expression was determined using a Taqman microRNA assay (Life Technologies, assay ID000379, 000391, 000397, 002623 and 001094 and ID001006). 1µL of eluted RNA was used for the reverse transcription reaction using the TaqMan micro RNA Reverse Transcription Kit (Life Technologies, 4366596) and 1.33µL of cDNA was used per 20 µL PCR reaction in triplicate using Taqman Universal Master Mix II (Life Technologies, 4440038). For messenger RNA gene expression, 1µL of eluted RNA was used for cDNA synthesis using a random primer (Life Technologies, 4368814), and 1.33 µL of the cDNA was used for a 20µL 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.

Results and Discussion

Size distributions of exosomes isolated from IMR-90 and Jurkat cells.

Nanoparticle Tracking Analysis (NTA) was used to confirm the size range of the isolated exosomes. Ultracentrifuge pelleted exosomes were diluted serially in increments of 10 (in 1X PBS, freshly sterile filtered through 0.01um nylon syringe filters several times) to reach an optimal particle concentration as measured by NTA in the range of 10^6 to 10^8 particles/mL. The size distribution of exosomes from IMR90 and Jurkat are presented in Figure 1, and cover the range of 30-100 nm as expected for exosomes.

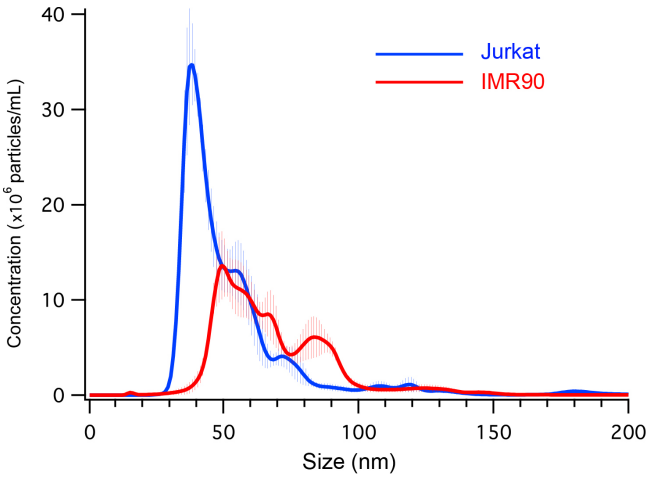


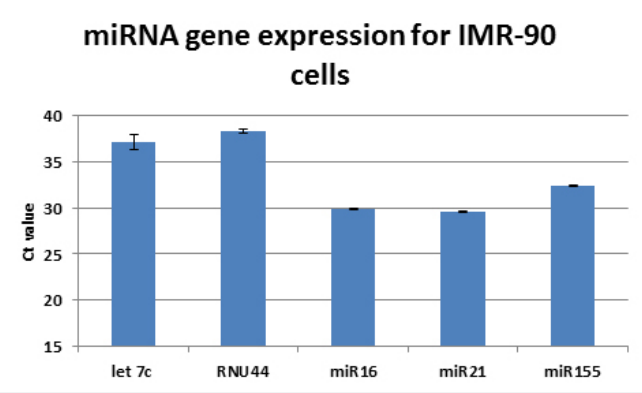
Figure 1: Size distribution of exosomes using Nanoparticle Tracking Analysis.

MiRNA gene expression data demonstrates that miRNA was successfully extracted from exosome samples.

1µL of eluted nucleic acid was used for let 7c, RNU44, miR16, miR21 and miR155 gene expression. The average cycle threshold (Ct) was calculated from triplicate samples. The average Ct values for let 7c, RNU44, miR16,miR21 and miR155 target gene expression in IMR-90 cells were 37.13+/-0.784, 38.32+/-0.21, 29.85+/-0.08, 29.60+/-0.05 and 32.39+/-0.06 respectively (Figure 2 Left). The average Ct values for let 7c, RNU44, miR16,miR21 and miR155 target gene expression in Jurkat cells were 36.97+/-0.63, 37.02+/-0.07, 27.52+/- 0.07, 33.37+/-0.38 and 35.29+/-0.37 respectively (Figure 2 Right). This result indicates that miRNA was successfully extracted from exosomes harvested from cell culture medium utilizing the RNAdvance Cell v2 kit. The results showed that miR16 was the most highly expressed genes and let 7c and RNU44 were lowest expressed genes in these exosomes.

RNA gene expression data demonstrates that messenger RNA was successfully extracted from exosome samples.

1µL of eluted nucleic acid was used for ACTB1, B2M, GAPDH and HPRT gene expression to determine messenger RNA was successfully



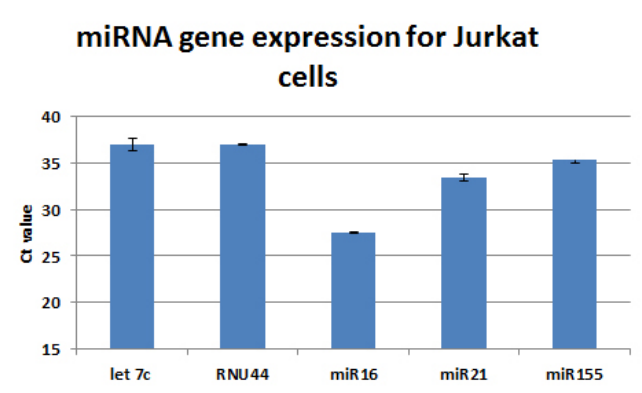
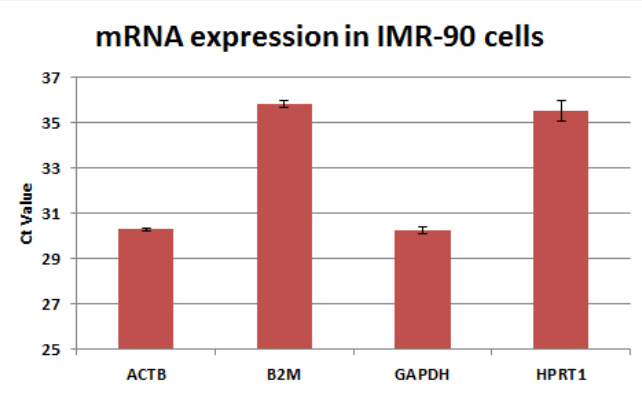


Figure 2: miRNA gene expression from cultured medium of IMR-90 cells (Left) and Jurkat cells (Right).



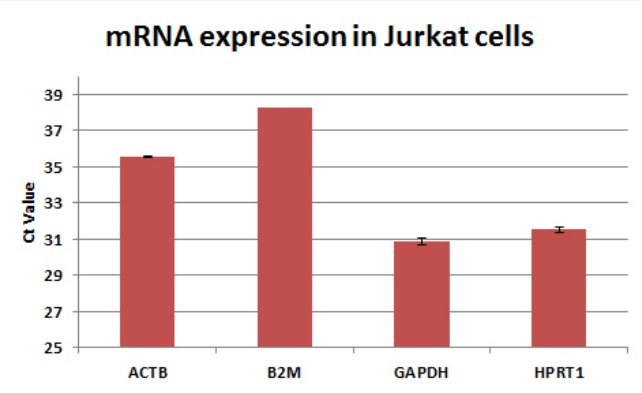


Figure 3: mRNA gene expression from cultured medium of IMR-90 cells (Left) and Jurkat cells (Right).

extracted from exosomes. The average cycle threshold (Ct) was calculated from triplicate samples. The average Ct values for ACTB, B2M, GAPDH and HPRT1 gene expression in IMR-90 cells were 30.27±0.075, 35.79+/-0.157, 30.25+/-0.161 and 35.50+/-0.442 respectively (Figure 3, Left). The average Ct values for ACTB1, B2M, GAPDH and HPRT1 gene expression in Jurkat cells were 35.53±0.016, 38.30, 30.86+/-0.187 and 31.51+/-0.179 respectively (Figure 3, Right). This result indicates that messenger RNA was successfully extracted from exosomes harvested from a cultured medium of cell cultures utilizing the RNAdvance Cell v2 kit. Among these genes, B2M has the lowest amount detected and GAPDH showed the highest expression in both cell lines.

Conclusions

The data from this study shows that the Beckman's RNAdvance Cell v2 Kit can be used for miRNA and RNA extraction from exosomes The magnetic bead based extraction protocol provides scalable throughput and it is automation-friendly.

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