

Comparison of Methods for miRNA Extraction from Plasma and Peripheral Blood Mononuclear Cells

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miRNAs are small non-coding RNA that are known to regulate gene expression at transcription level. Altered expression levels of miRNAs due to the infections can serve as clinically relevant biomarkers. Reproducible and efficient recovery of miRNA from biological samples is important for their reliable quantification. Therefore, we compared the recovery of miRNAs from plasma and PBMC using several commercially available RNA isolation kits in the presence and absence of carrier molecules to enhance the yield, by quantification of hsa-mir-103-5p, hsa-let-7e and hsa-mir-30b-5p with RT-qPCR. Organic extraction and precipitation of total RNA with or without the addition of tRNA from brewer's yeast or glycogen as carrier molecules, mirVana microRNA isolation kit (Applied Biosciences), and miRNeasy Serum/Plasma Kit (Qiagen) with or without tRNA were evaluated for RNA recovery from plasma. mirVana kit and miRNeasy kit were also evaluated for RNA recovery from PBMC. RNA isolations were performed from either plasma or PBMC isolated from whole blood collected from healthy volunteers with informed consent. Total RNA was used for subsequent 3' polyadenylation of the miRNA followed by cDNA synthesis. Presence of target miRNAs in plasma and PBMC were confirmed by RT-qPCR using target specific primers. Primer specificity was confirmed using NCBI blastn suite. All three miRNA targets were detectable in PBMC using the two commercial kits, without the addition of a carrier molecule. PBMC samples processed with miRNeasy extraction kits showed earlier target amplification due to concentration of total RNA in smaller elution volumes compared to the mirVana extraction method. Addition of low amount of carrier RNA (1 µg/mL) yielded more RNA. Adding high amount of carrier RNA (10 µg/mL) during RNA extraction with mirVana kit and organic extraction showed selective effect on RNA recovery. Using glycogen as the carrier for organic extraction also yielded higher amount of miRNA from plasma. Therefore, addition of limited amount of carrier molecules can enhance the miRNA recovery.

Keywords: microRNA extraction, PBMC, Plasma, Carrier molecule

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