Sample collection

SpeciMAX Saliva Collection Kits stabilize miRNA in saliva samples

Introduction

Saliva is a biofluid that can be noninvasively collected and enables research in a variety of areas including oncology. Oncology researchers have published research documenting how cancer influences biomarkers of salivary microRNAs (miRNAs) by altering their genetic expression [1]. For example, pancreatobiliary tract cancer is a highly fatal cancer characterized by the occurrence of a malignant carcinoma in pancreatic, gallbladder, and extrahepatic bile ducts. Detection of pancreatobiliary tract cancer is difficult because it lacks typical clinical symptoms and because of its anatomical location. Biomarkers in salivary exosomes, like those in sera, can be useful for research in cancer screening and detection [2]. Thermo Fisher Scientific has developed the Applied Biosystems™ SpeciMAX™ Saliva Collection Kits that collect, store, stabilize, and transport saliva safely. Total RNA from saliva can be extracted and utilized in various downstream application workflows, such as next-generation sequencing (NGS), real-time PCR using the Applied Biosystems™ TaqMan™ OpenArray™ Human MicroRNA Panel, and microRNA analysis using TaqMan™ MicroRNA Assays on the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System.

The Applied Biosystems™ MagMAX™ *mir*Vana™ Total RNA Isolation Kit enables extraction of true total RNAs, including small RNAs from saliva collected using the SpeciMAX kits, and provides workflow solutions with automatable magnetic-bead technology suitable for low- and high-throughput sample processing. The MagMAX *mir*Vana kit can also be used to isolate total RNA from serum, plasma, whole blood, tissue, cell culture, and urine specimens.

We evaluated the stabilization of salivary endogenous and mimic miRNAs in SpeciMAX collection kits utilizing the MagMAX *mir*Vana Total RNA Isolation Kit sample preparation workflow on the Thermo Scientific™ KingFisher™ Apex system (Figure 1). The quantity and quality of the miRNA recovered was evaluated by yield and real-time PCR performance.

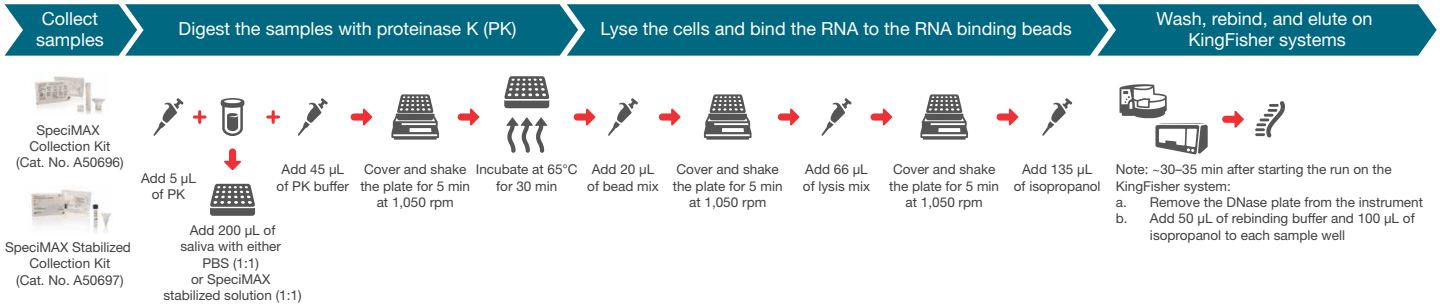

Figure 1. Workflow for processing saliva for the isolation of total RNA, including small RNAs such as microRNAs, from a wide variety of sample types using automated KingFisher purification systems.

Methods

In this study, we evaluated the extraction of miRNAs from saliva using the MagMAX *mir*Vana kit, which isolates total RNA from serum, plasma, whole blood, tissue, cell culture, and urine specimens. We demonstrated the use of saliva as a sample matrix with the currently published (blood sample) workflow, and incorporated a modified, larger 200 µL sample and 45 µL PK digestion buffer volume inputs, as opposed to the published volume inputs. We used the published biofluids script on the KingFisher Apex system to accommodate the saliva sample or the larger input volume.

Raw saliva was collected from 6 healthy donors. Saliva from each donor was split into two aliquots. Each aliquot was combined with either PBS (nonstabilized) or SpeciMAX™ stabilizing solution (stabilized) in a 1:1 ratio. The samples were then evaluated for the stability of miRNA targets in their respective conditions. The mixtures were vortexed and the homogeneous saliva mixture was further split into aliquots for each of the 8 time points tested (16 aliquots per donor: 8 controls and 8 tests). Each sample was then contrived with miRNA pre-amp precursor mimic spike-in, hsa-miR-4644, which concentrated at 0.050 ng/µL incorporated an overall number of 300 copies per extraction. The pre-amp precursor hsa-miR-4644 is designed to mimic endogenous mature miRNAs to study the functions and mechanisms of endogenous miRNAs in oncology disease research [3]. A negative or no-mimic control for both the PBS and SpeciMAX solutions was included for each saliva donor at each time point, to serve as a background comparison for all time points (data not shown). Additionally, we included mimic-only samples (for both PBS and SpeciMAX solutions) in tubes with no saliva, to ensure sterility of the solutions (data not shown). All samples were subjected to a reverse time course study. At the selected time points, samples with SpeciMAX solution were stored at ambient temperature, 15°C to 30°C, and PBS samples were stored refrigerated at 2°C to 8°C. All nonstabilized and stabilized saliva samples were stored per the specifications of the SpeciMAX raw and SpeciMAX stabilized collection kits, respectively. Endogenous salivary miRNA targets hsa-let-7b and hsa-miR-16, and pre-amp precursor mimic hsa-miR-4644, were evaluated after storage for >95 hr, 48–72 hr, 24–36 hr, 3–4 hr, 1–1.5 hr, 0.5–0.75 hr, and 0 hr (control) prior to extraction.

The extracted miRNA was quantified using Invitrogen™ Qubit™ microRNA Assay Kits. Applied Biosystems™ TaqMan™ MicroRNA Assays were used to determine compatibility with downstream analysis. The TaqMan™ MicroRNA Reverse Transcription Kit, TaqMan™ Fast Advanced Master Mix, and TaqMan MicroRNA Assays were used for the detection of hsa-miR-4644, hsa-let-7b, and hsa-miR-16 on the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System (384-well format). Results were analyzed using QuantStudio™ Real-Time PCR Software.

Results

Extracted yields measured using the Qubit microRNA Assay Kit indicated donor-to-donor variability in the miRNA recovered from both nonstabilized (PBS) and stabilized (SpeciMAX solution) saliva samples (Figure 2). Mean yields from the nonstabilized saliva samples in PBS were higher at all time points for all donors with the exception of donor 3, whose samples yielded lower miRNA concentrations from both nonstabilized and stabilized samples at all time points evaluated in this study. The higher yields from nonstabilized samples are likely due to higher bacterial loads when compared to stabilized samples. The SpeciMAX solution has a virus-inactivating stabilization agent that is likely also preventing excess bacterial growth.

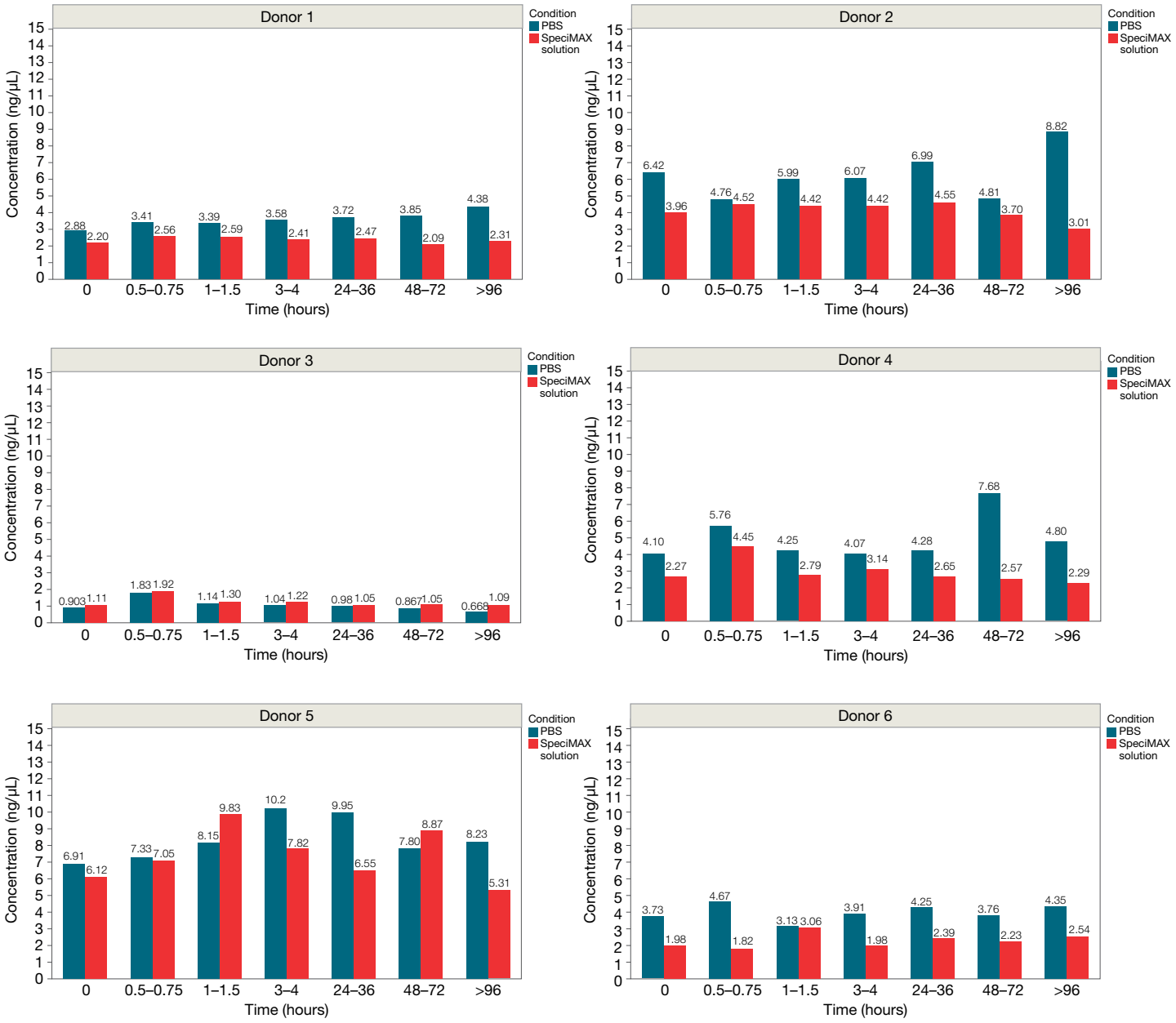

Figure 2. miRNA yields from six stabilized and nonstabilized saliva samples after various storage times. Saliva samples were processed using the MagMAX *mir*Vana Total RNA Isolation Kit on the KingFisher Apex system using the biofluids script. Nonstabilized samples in PBS and stabilized samples in SpeciMAX solution were evaluated for microRNA concentration with the Qubit microRNA Assay Kit after various storage times.

Overall, RT-qPCR detection of endogenous miRNA targets, hsa-miR-16 and hsa-let-7b, showed a ΔC_t shift of <1 at each time point for both nonstabilized and stabilized samples. The ΔC_t shift was determined by subtracting the C_t values for each time points from the control time point (0 hr) (Figure 3A). This evaluation indicates that the endogenous miRNAs were detected from the moment of collection and all throughout the study. The mimic, hsa-miR-4644, was stable and produced detectable C_t values across all time points for all samples in SpeciMAX stabilizing solution.

The nonstabilized saliva samples in PBS containing hsa-miR-4644 had a positive ΔC_t shift, which indicates the mimic was not as stable throughout the study (far-right panel of Figure 3B). RT-qPCR results showed no detection of endogenous miRNA targets, hsa-miR-16 and hsa-let-7b, for tubes with the mimic only, while hsa-miR-4644 amplified as expected (data not shown).

A

C_t values of samples at the 0 hr time point						
Donor	hsa-let-7b		hsa-miR-16		Mimic hsa-miR-4644	
	PBS	SpeciMAX stabilizing solution	PBS	SpeciMAX stabilizing solution	PBS	SpeciMAX stabilizing solution
1	29.80	27.71	31.02	28.71	19.01	18.80
2	29.14	29.19	28.92	29.85	19.20	19.58
3	30.79	29.18	31.04	28.84	19.13	18.84
4	29.66	27.25	30.83	30.24	19.08	18.53
5	28.99	26.91	28.27	26.22	19.41	19.26
6	29.25	29.03	29.08	28.14	19.44	19.28

B

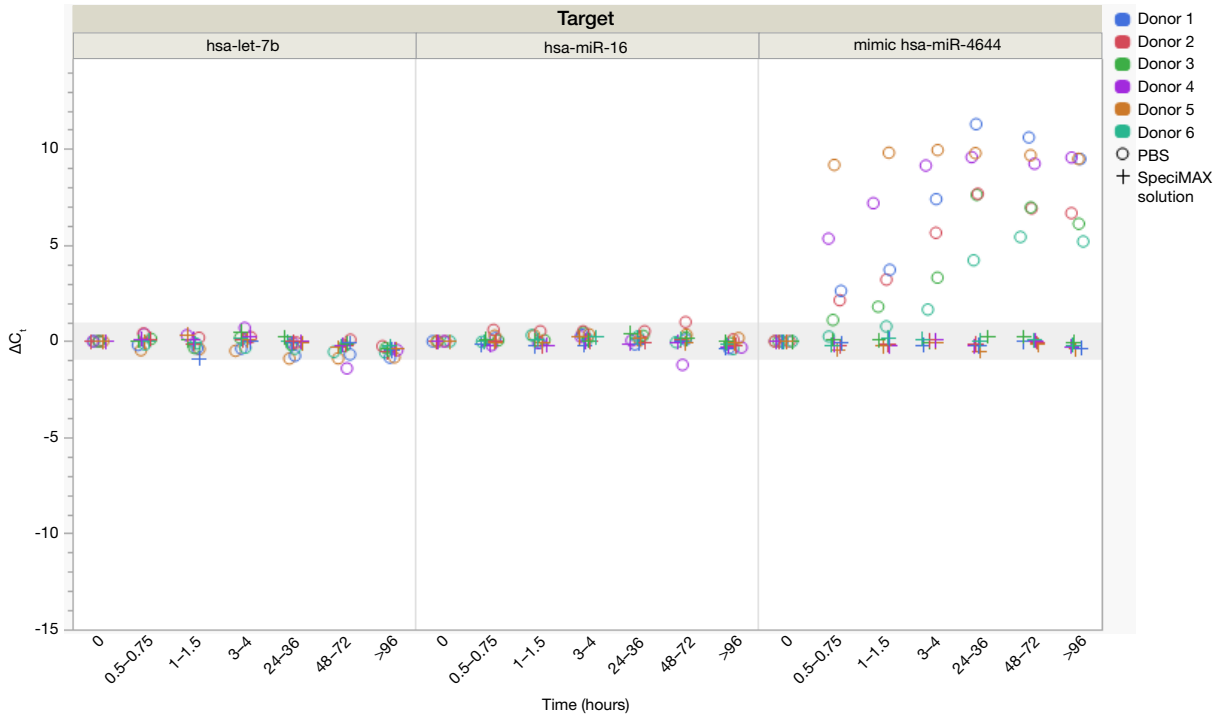

Figure 3. RT-qPCR results for endogenous miRNA targets, hsa-miR-16 and hsa-let-7b, and mimic hsa-miR-4644. (A) C_t values for both nonstabilized saliva samples in PBS and stabilized saliva samples in SpeciMAX stabilizing solution at the 0 hr time point. Note the generally lower C_t values for samples stabilized in SpeciMAX solution. **(B)** ΔC_t shifts of all samples at various time points for donor samples in PBS (circles) or SpeciMAX solution (pluses). The mimic results indicate that the mimic was not as stable in the saliva samples in PBS as compared to the saliva samples stabilized with the SpeciMAX stabilizing solution.

Conclusions

SpeciMAX Saliva Collection Kits can be used to collect, store, stabilize, and transport saliva safely without refrigeration or freezing. Endogenous and spiked mimic miRNAs can be detected in saliva samples stabilized with the SpeciMAX stabilizing solution, with C_t values shifting by <1 over the >96-hours evaluated in this study. The SpeciMAX Stabilized Saliva Collection Kit, paired with the MagMAX *mir*Vana Total RNA Isolation Kit on the KingFisher Apex system, offers an end-to-end workflow from sample collection and stabilization to total RNA purification. The yield and quality of miRNAs extracted from saliva samples in this study indicate that the demonstrated workflow is suitable for obtaining high-quality miRNA for a variety of research applications.

Author

Michelle Leija, Thermo Fisher Scientific, Austin, TX

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References

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Ordering information

Product	Quantity	Cat. No.
MagMAX *mir*Vana Total RNA Isolation Kit	96 reactions	A27828
SpeciMAX Stabilized Saliva Collection Kit	100 kits	A50697
KingFisher Apex Benchtop Sample Prep System	1 each	5400940
QuantStudio 7 Flex Real-Time PCR System, 384-well, desktop	1 each	4485701
TaqMan MicroRNA Reverse Transcription Kit	200 reactions	4366596
TaqMan MicroRNA Assay	50 RT/150 PCR reactions	4427975
Qubit microRNA Assay Kit	500 assays	Q32881

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