New RNA GeneFix Saliva Collector produces High Quality stable RNA

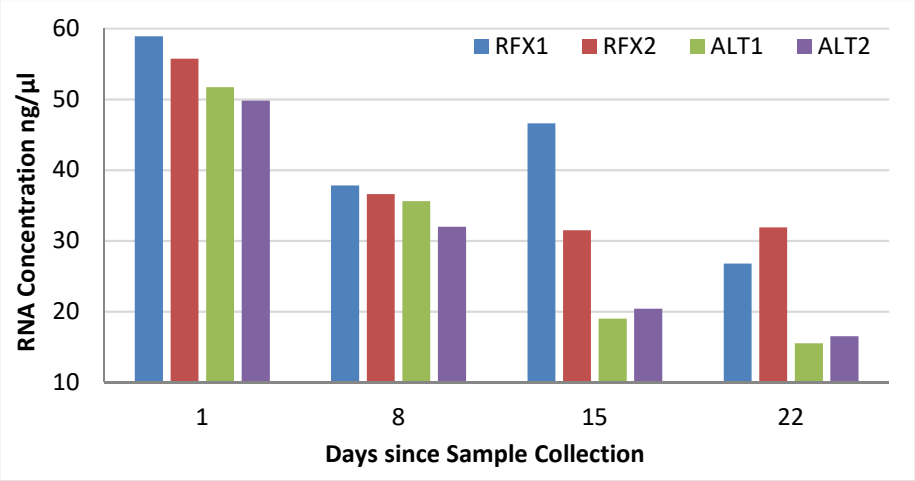
In recent years the study of gene expression has come to be as important as gene sequence, particularly when looking at disease and microbiome.

Many RNA's are fleeting and unstable molecules, quickly broken down by ubiquitous enzymes, and degrade rapidly. RNA collection and analysis is especially challenging from Saliva samples given the high enzyme quantities found in the oral cavity. Isohelix has developed a unique non-toxic RNA saliva stabilization buffer, which immediately preserves the RNA and uses the successful GeneFiX™ collection system for quick and easy sampling.

In this study, we collect saliva samples from several adult volunteers into sterile tubes, vortexed them for homogeneity, and divided them equally into GeneFiX™ RNA collectors (RFX), and an alternative brand collector, according to the manufacturer's instructions. The samples were stored at room temperature, with 300µl vortexed aliquots taken from each at 1, 8, 15 and 22 days post collection into RNAse-free 2ml tubes, and, following the manufacturers RNA precipitation step, RNA was isolated using Quick-RNA mini prep from Zymo. The RNA was analysed by Qubit, Nanodrop, qPCR and Agarose gel electrophoresis. The results shown are from selected individuals, or mean results from the group.

Results

Qubit™ RNA Assay demonstrates superior yields and stability:



- Concentration and yields were higher in RFX stabilized samples than the alternative collector.
- RFX samples remain stable for longer, giving a significant advantage when storing 15 days and beyond.

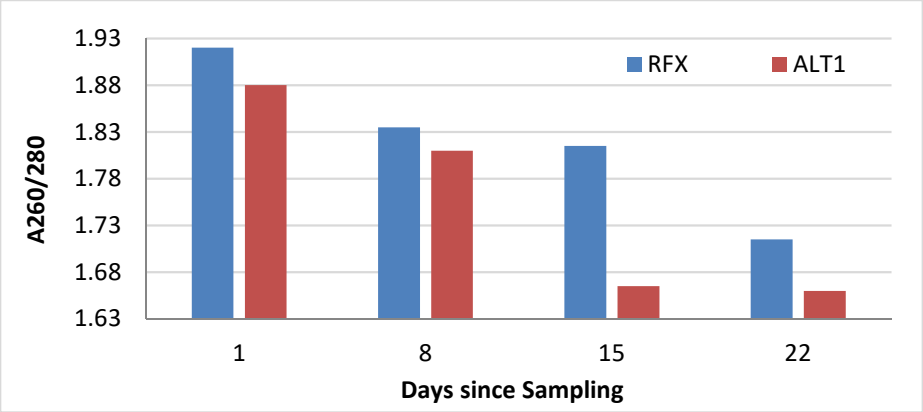
Difference in Cq Day 1 to Day 22

Saliva	ALT	RFX
1	3.67	1.66
2	4.83	1.93

Real-Time PCR Performance

qPCR data shows that the Cq value change from Day 1 to Day 22 is considerably lower for RFX over the alternative collector, indicating that amplifiable mRNA in samples remains stable for longer in RNA GeneFiX™ buffer.

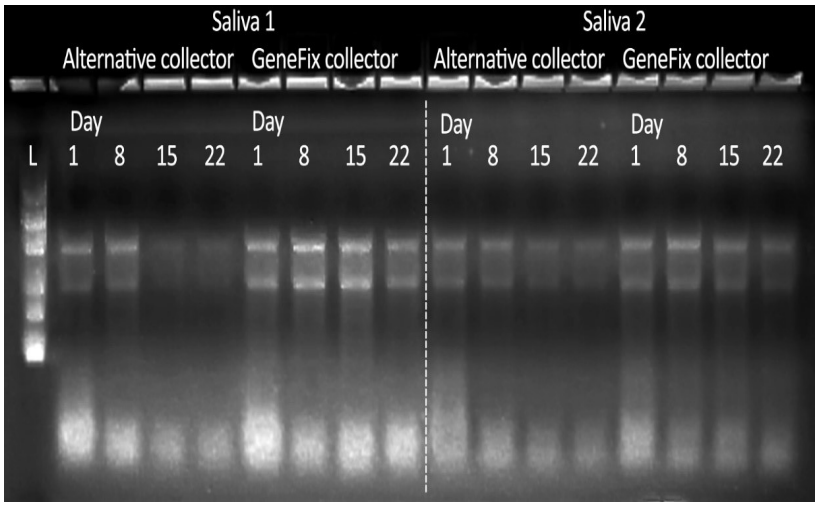
Absorbance Ratio Shows RFX Maintains Sample Purity:



- Sample purity is higher in RFX samples at all sampling points, and remains stable for longer in comparison to the alternative collector.

2.2% Agarose Lonza FlashGel – Marker sizes 0.5-9.0 Kb:

- All samples show two clear bands of Ribosomal RNA and a bright band of smaller species <500bp. The brightness of the bands falls over time, indicating less RNA.
- RFX bands maintain a greater brightness, indicating greater stability over time in comparison to the alternative.



Conclusions

- **This study upholds Isohelix's claim of high performance RNA stability in the GeneFiX™ RNA Saliva Collector for at least 14 days and often maintained beyond this point.**
- **The RFX purity, concentration and yield are higher over time compared to the alternative.**
- **The collection method is simple, ideal for remote use, with ample time to transport or to bundle large sample numbers for processing. An RNA precipitation reagent is supplied, making the collector compatible with any RNA isolation kit currently available.**
- **The buffer is non-toxic, which simplifies risk assessment and adds additional protection to patients, as does the design patented funnel, which protects against spills.**
- **The RNA isolated is of sufficient yield and purity for most downstream applications, including oral microbiome studies, and gene expression.**