Swift & Simple Purification of Total RNA from Oral Samples using the NEW Isohelix *Xtreme*-RNA Isolation kit

Introduction

Due to current events, the demand and importance of high-quality total RNA from oral samples has become paramount. To meet this, Isohelix has launched the new *Xtreme*-RNA (XMR) Isolation kit; a rapid, simple to use purification option for stabilised saliva and buccal swab samples utilising a novel enzymatic method of RNA purification, free of highly toxic components that are common to the vast majority of RNA purification kits available.

To demonstrate the high performance of the *Xtreme*-RNA kit, this study compares the extraction of GeneFix RFX-01 saliva collectors using the XMR with a commonly available RNA extraction kit, *Kit Z,* by contrasting sample yield, purity, and rt-qPCR performance using multiple mRNA gene targets.

Methods

Five RFX-01 saliva samples were collected from individual donors. The samples were mixed into a single homogenised solution and vortexed thoroughly to generate a stock sample, eliminating donor variation so that differences in the performance between the two kits could be more accurately determined. Using this, 10 x 250µl aliquots were prepared and were separated into two sets of five; one set to be purified using the *Xtreme*-RNA kit, the other set purified using *Kit Z,* following each kit's respective protocol. The final elution volume of the samples was 50µl in RNAse-free H_2O. Following purification, samples were assessed for purity (A260/280, A260/230) using an Implen N60 spectrophotometer, and for yield using the Qubit BR RNA assay.

In addition, the integrity of sample mRNA was evaluated by rt-qPCR, targeting three genes (ACTB, GAPDH, & RPS18) using 10ng of extracted RNA samples in a 10µl reaction using the Bio-Rad iTaq™ Universal SYBR® Green One-Step Kit, run on a CFX Connect thermal cycler following the kit's protocol. Prior to rt-qPCR analysis, samples were DNAse treated with Invitrogen DNAse I to remove contaminating DNA from samples. Appropriate controls (No Template controls and No Reverse Transcriptase controls [NTCs/NRTs]) were used to determine residual gDNA contamination.

Results & Discussion

Yield & Purity Assay:

- Yields and purities of all the samples tested were high *(Table 1),* with concentrations greater than 70 ng/µl, with average A260/280's of 2.0 & A260/230's of >1.7, exemplifying how high-quality total RNA is purified from GeneFix Saliva collectors.

- Averages between the two extraction methods were comparable, demonstrating the performance of *Xtreme*-RNA alongside other popular RNA extraction kits.

Sample ID	Qubit RNA Conc (ng/µl)	A260/A280	A260/A230
Isohelix (A)	69.5	2.0	1.7
Isohelix (B)	79.9	2.0	1.7
Isohelix (C)	75.6	2.0	1.8
Isohelix (D)	68.1	2.0	1.7
Isohelix (E)	58.5	2.0	1.8
Isohelix Mean	**70.3**	**2.0**	**1.7**
Kit Z (A)	66.4	2.0	1.8
Kit Z (B)	76.8	2.0	1.5
Kit Z (C)	72.2	2.0	1.9
Kit Z (D)	64.4	2.0	1.9
Kit Z (E)	77.7	2.0	1.7
Kit Z Mean	**71.5**	**2.0**	**1.8**

Table 1: Qubit BR RNA assay & Implen Photometer data comparing yield and purity between the two sample sets.

rt-qPCR Assay:

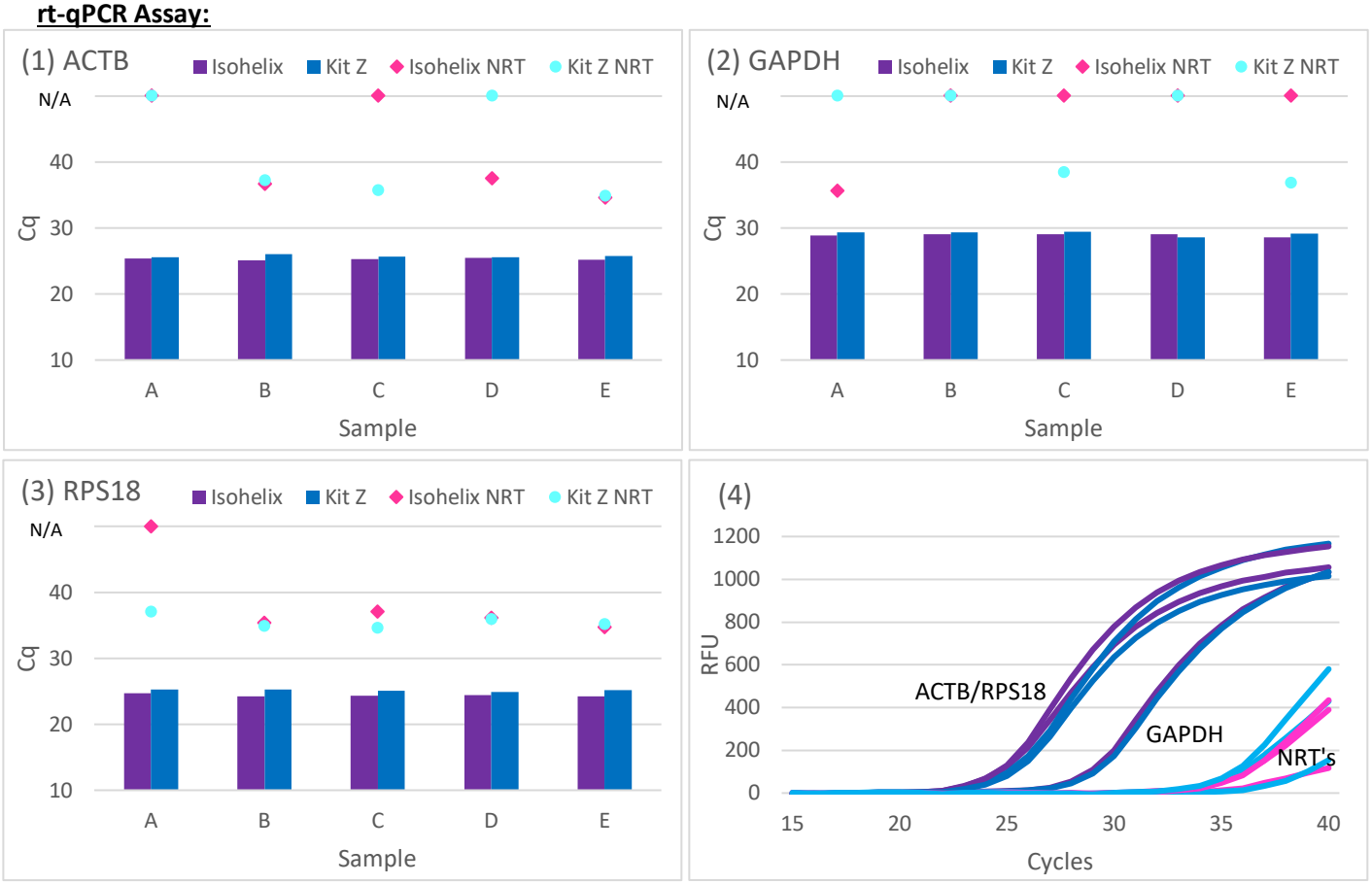

Figure 1: rt-qPCR amplification data comparing performance between Xtreme-RNA & Kit Z*. These data compare cycle thresholds (Cq's) between XMR & Kit Z for the target genes (1) ACTB (2) GAPDH & (3) RPS18, with accompanying NRT controls to determine true mRNA signal. NRT'S with a 'N/A' value indicate no residual DNA present. (4) Amplification curves generated using the averaged data from each sample set.*

- Samples purified using the *Xtreme*-RNA isolation kit generated Cq signals comparable to, or in most cases, lower (better) than equivalent samples isolated using *Kit Z (Figure 1),* which indicates the presence of high-quality mRNA that can be readily detected and amplified.

- Differences in Cq between samples and their equivalent NRT controls were high, confirming that signals obtained were truly from mRNA and not DNA. On average, it was calculated that DNA contribution to the amplification signal of samples was < 0.1%.

Conclusions

- **The Isohelix *Xtreme*-RNA Isolation kit is a viable alternative to other available kits on the market, with comparable yields and purities, and on average superior results when running rt-qPCR amplification on salivary total RNA samples and subsequent expression analysis.**

- ***Xtreme*-RNA is incredibly simple to use and can be used to fully purify samples within an hour.**

- **Unlike most other methods, Xtreme-RNA is free of toxic and costly reagents commonly used in RNA extraction kits, allowing for easy and safe disposal following its use.**