

Isoheli swab: Stabilisation of RNA samples for up to 96 Hours

Methods:

Three swab samples were collected from separate donors (N, R, & A) into Isoheli BuccalFix, following the kit collection instructions. Samples were then incubated at room temperature (23°C ± 5°C). To test the stability of RNA in BuccalFix, 300µl aliquots of each sample were extracted using a column RNA isolation kit at the following time points after incubation: 0 Hours (Fresh), 48 Hours, & 96 Hours.

Following extraction, samples were analysed for sample yield, purity, & integrity using Qubit RNA assay, and Nanodrop assay. In addition, SYBR Green rt-qPCR reactions were run using 10ng of each sample targeting the human ACTB gene, to assess suitability of collected samples for downstream applications.

Results:

Nanodrop/Qubit Assay:

Fresh	N	R	A	Mean
Nanodrop RNA Yield (ng/ul)	18.66	36.28	55	36.65
Qubit RNA Yield (ng/ul)	47.9	74.7	80.7	67.8
A260/280	2.00	2.10	2.02	2.0
A260/230	1.75	1.96	1.38	1.7

48h	N	R	A	Mean
Nanodrop RNA Yield (ng/ul)	13.23	15.96	18.22	15.80
Qubit RNA Yield (ng/ul)	16	28.7	43.5	29.4
A260/280	1.62	1.87	2.03	1.8
A260/230	0.95	1.77	2.13	1.6

96h	N	R	A	Mean
Nanodrop RNA Yield (ng/ul)	12.84	20.71	33.66	22.40
Qubit RNA Yield (ng/ul)	21	33.3	59.9	38.1
A260/280	1.83	2.12	2.02	2.0
A260/230	0.31	1.01	1.26	0.9

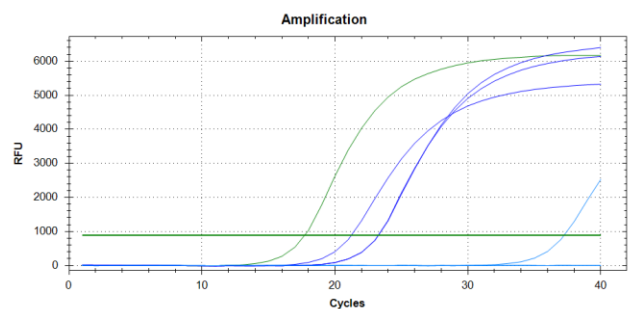
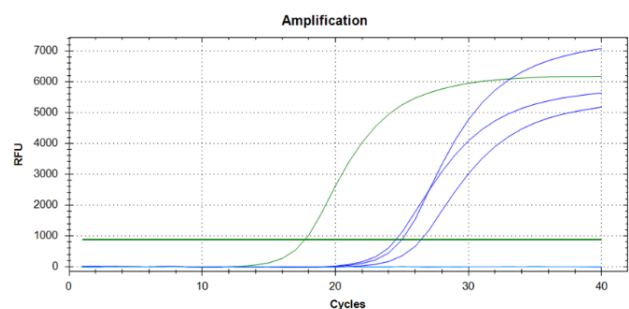
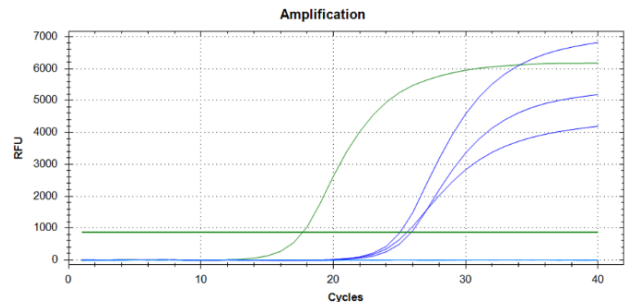
- Qubit RNA & Nanodrop analysis show that swab total RNA samples stabilised using BuccalFix maintain yield, purity, and sample integrity stored at room temperature up to 96 hours following collection.

ACTB rt-qPCR Assay:

BuccalFix RNA 96h stability testing

ACTB	+RT Cq	-RT Cq	ΔCq
10ng RNA Std	17.70	27.45	-9.75
N Fresh	25.58	N/A	-
N 48h	25.91	N/A	-
N 96h	25.06	N/A	-
R Fresh	24.52	N/A	-
R 48h	26.43	N/A	-
R 96h	25.01	N/A	-
A Fresh	21.21	37.22	-16.01
A 48h	23.25	N/A	-
A 96h	23.24	N/A	-

- ACTB rt-qPCR of swab/BuccalFix collected RNA samples reported consistent amplification of the target gene.
- Signal from each donor sample was consistent, and did not degrade over the 96-hour incubation period.



Conclusions:

- RNA samples collected from swabs and stored in BuccalFix are stable for up to 96 hours following collection.
- Stabilised samples produce reliable, repeatable results during downstream rt-qPCR analysis.