

Arcis Saliva Nucleic Acid Extraction Protocol

Saliva is increasingly used as a bio-fluid for rapid diagnostics. Sample collection is non-invasive and easy to administer and there are several commercial collection kits on the market. Here we look at the release of DNA from saliva samples following Arcis treatment and assess the DNA for quality, quantity and compatibility with qPCR. Furthermore, the saliva samples will be assessed for stability in Arcis reagent 1, with samples stored at room temperature and at 40°C for real-time and accelerated stability testing.

Sample Collection

Donors rinsed their mouths with water 10 minutes prior to providing samples. Donors provided samples of approximately 2ml of saliva into 15ml Falcon tubes. These samples were vortexed mixed prior to testing

Storage conditions

Tubes are shipped and stored at room temperature. Samples which have been lysed in buffer 1 are stable at room temperature for 30 days.

Materials provided

Materials provided	Quantity	No. Reactions
Tube 1 Lysis Buffer	1	48
Tube 2 Wash Buffer	1	48

Method

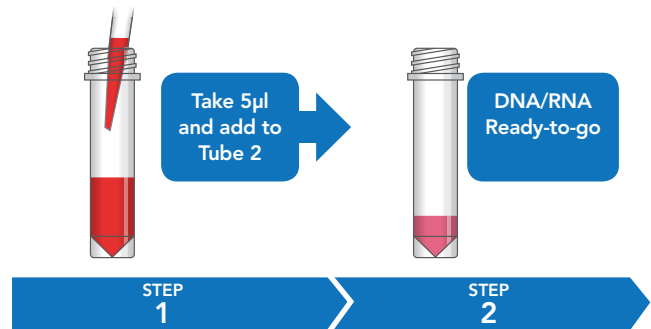
Protocol	Volume of Saliva (µl)	Volume of Reagent 1 (µl)	Ratio of Tube 1: Tube 2
1 (Standard)	30	170	1:4
2	60	140	1:4
3	90	110	1:4
4	30	170	1:2

- Samples of saliva were added to reagent 1 according to the table above
- The sample was then incubated for 1 minute at room temperature. After this 5µl was transferred to Tube 2 (see ratios) and mixed thoroughly.
- 5µl of the resultant solution was then added to the PCR master mix for a final reaction volume of 25µl

PCR conditions

Initial denaturation 95°C 10 min, denaturation 95°C 15 sec, annealing 60°C 60 sec 45 cycles on LC480 II Lightcycler. Fluorescence readings acquired in the VIC (540-580nm) at the annealing step.

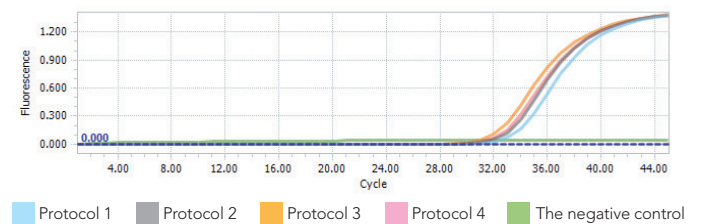
	Vol µl	Vol µl
ABI Master Mix	12.5	750
F Primer (50µM)	0.4	24
R Primer (50µM)	0.4	24
Probe (1µM)	0.5	30
Water	6.2	372
Dispensed per well	20	20
Template (tube 2)	5	5



Results (Ct values averaged)

	1	2	3	4
	32.65	32.02	31.24	31.70
	31.13	32.55	31.50	31.63
	32.45	32.36	32.55	33.08
	31.85	32.12	32.40	33.06
	33.75	32.49	31.78	33.01
	31.56	31.80	31.99	32.91
Average	32.23	32.22	31.91	32.57
SD	0.93	0.29	0.51	0.70

Amplification Curves



Conclusion

All protocols yielded positive results, indicating that DNA extraction from saliva samples is possible with Arcis reagents. There is little difference in Ct value or amplification between the protocols, indicating robustness of the system with a wide working range of volumes.