Arcis Buccal Swab Nucleic Acid Extraction Protocol

Buccal cell usage has been shown by many to be a cost effective and safe method to isolate DNA for various biological experiments especially large epidemiological studies, genetic ID testing and forensic analysis. Non-invasive DNA collection methods are preferred over phlebotomy in order to increase study participation and compliance in research centres and for sick patients in hospital settings. Here we describe a protocol using the Arcis DNA Blood Kit - a ready to use, room temperature kit comprising two reagents enabling pre-analytical processing of samples. In 3 minutes, with no prior sample preparation, the kit allows you to go from swab to downstream nucleic acid investigations without the need for isolation or purification. The kit is suitable for untreated specimens and specimens that have been frozen. The DNA released is ready for immediate use in PCR or other molecular applications. The Arcis DNA Blood Kit is intended for in vitro identification of nucleic acids or diagnostic use.

The following protocol describes the extraction of DNA from buccal swabs collected from two donors (10 scrapes per cheek) and the subsequent analysis of the genetic material extracted by the Arcis DNA Blood Kit.

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

Samples

For best results rinse mouth with water immediately prior to sampling. Samples tested were from two donors (10 scrapes per cheek), swab was rubbed firmly against the cheek. The swab was then agitated in the lysis buffer for 60 seconds.

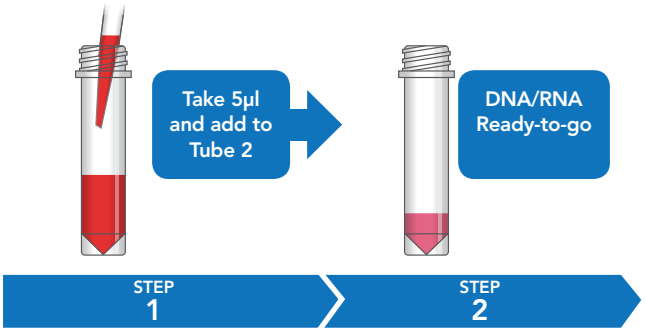
Method

- Post scrape swabs were dropped into the buffer 1 Lysis solution (150µl) and agitated briefly to release the nucleic acids by rotating 10 times and squeezing against the sidewalls of the tube. Swab is then snapped at breakpoint before leaving the swab in the tube. Tubes were then vortexed.
- The sample was then incubated for 1 minute at room temperature. After this 5µl was transferred to Tube 2 (20µl) 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 channel (540-580nm) at the annealing step.

hRNase P PCR Mix	Vol µl
Master Mix 2x	12.5
F Primer (50µM)	0.4
R Primer (50µM)	0.4
Probe (1µM)	0.5
Water	6.2
Template (tube 2)	5



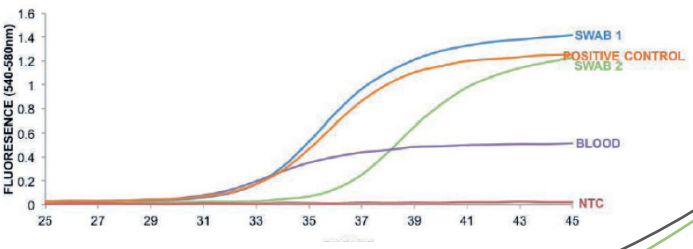
Results (Ct values averaged)

	Donor 1	Donor 2
Positive control	32.59	32.49
Swab 1	31.94	32.88
Swab 2	35.5	35.62
30µl blood	30.53	30.37
NTC	No amp	No amp

Conclusion

The protocol can successfully extract amplifiable DNA from buccal swabs. For swab 1 the Ct values for the hRNaseP assay are between 31.94 and 32.88, the Ct values for swab 2 are later showing the heterogeneity between samples. All swab PCR curves were prominent and easily detected.

Buccal Swabs



Version 001 03/17

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