Application Note: SGC/SK1-03

DNA stability on Isohelix SK1 buccal swabs stored with Isohelix Dri-Capsules is maintained over a 3+ year period.

Introduction:

DNA buccal swabs provide a convenient, cost-effective alternative to invasive venopuncture for the collection of DNA for genotyping, diagnostics, epigenetic studies, paternity, forensic and population studies as well as veterinary genotyping and diagnostics.

Other advantages of DNA buccal swabs include their use by untrained individual clients, patients or owners, as well as qualified professionals in clinics or hospitals.

DNA stability on swabs has been well documented and allows for sufficient time for the swab to be returned to the laboratory within a short to medium period, without undue breakdown of the DNA. If however the swabs need to be stored for longer periods prior to being returned to the lab, then storage at -20^{0}C has generally been considered the method of choice. In other situations where no freezer facilities are available, other methods such as air drying or using stabilising solutions are frequently used. Air drying is considered easy to use but is considered time consuming and has the disadvantage of potential contamination during the drying phase.
The use of stabilising solutions (like Isohelix DSK) does provide extremely long term stability (3 ½ + years), however it is advised that due to the nature of the components used, the kits should be handled by trained personnel.
We propose that the use of silica gel capsules (Isohelix type SGC/SK1) can be used as a viable alternative, offering reduced handling times and risks of cross contamination whilst providing a simple and effective way of stabilising long term, the DNA samples on the swab prior to extraction.

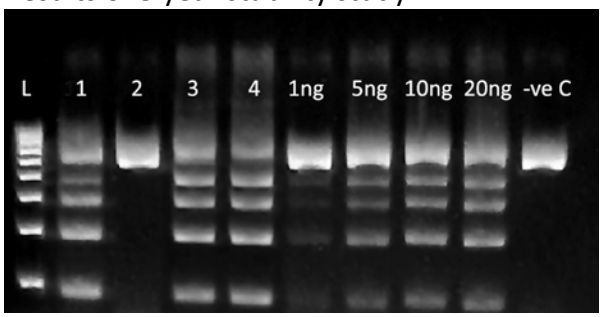
Method:

As described previously (see application notes SGC/SK1-01, multiple swabs were taken from individuals using Isohelix SK-1 swabs and stored in their tubes at room temperature with an Isohelix Dri-Capsule for varying lengths of time up to a maximum period of 36 months prior to analysis.

The DNA was isolated from the swabs using the Isohelix DDK DNA Isolation Kit. The quality of the extracted DNA was assessed using the Isohelix DQC-50 PCR Kit which is a multiplex PCR reaction specifically designed to check the quality and presence of human DNA in the extracted samples.

The DQC kit is designed to produce fragment sizes of 100, 200, 300, 400, 500 and 600 bp. If all 6 fragments are observed, the DNA is not denatured, fewer than 6 bands indicates the DNA is partially degraded. The 500bp fragment is an internal control derived from Lambda DNA, and should always be present even in negative controls, to show that the PCR reaction has been successful.
A range of human genomic DNA controls was also used to enable the yield of DNA from the swabs to be estimated by comparing band intensities with those of the known standards.
Degradation of the DNA on swabs stored at room temperature without Dri-Capsules or other methods of stabilisation has been documented previously. See application note SGC/SK1-01.

Results of 3 year stability study:

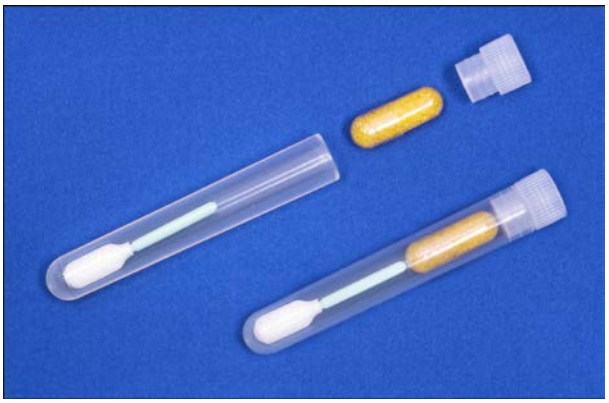


L = 100bp ladder
1 = DDK isolated SK1 swabs stored with Dri-Capsule for 3 years @ r.t.
2 = Clean, unused SK1 swab
3 = Freshly swabbed SK1 swab, person A
4 = Freshly swabbed SK1 swab, person B
1ng, 5ng, 10ng and 20ng standards: Purified human genomic DNA (Promega)
-ve Control = 5µl molecular biology grade water

The results from the DQC PCR show that the DNA isolated from the swab stored for 3 years at room temperature with a Dri-Capsule is intact and not degraded. This indicates that the Isohelix Dri-Capsules can provide long term stability of DNA on buccal swabs prior to extraction, without the need for storage in a freezer or use of chemical stabilisers.

Conclusions:

For long term stability requirements the use of Isohelix SGC Dri-capsules with Isohelix SK-1 swab kits for buccal swab sampling proved to be a reproducible and viable alternative to other methods of stabilising DNA such as air drying, freezing and chemical stabilisation. This study clearly demonstrates that DNA stability can be substantially increased, for a minimum time period of 3 years.



SK-1 swab with tube and silica gel capsule

As well as increased storage times, the silica gel capsules used together with the SK-1 tubes have other significant advantages in reducing the risk of cross contamination between samples, by allowing the swab samples to be stored separately from one another in their own individually identifiable tubes immediately after sampling. This is an important factor when the isolated DNA is likely to be used in downstream procedures such as PCR where contamination of the DNA is to be avoided.