

Long Term DNA stability data from studies using Isohelix Dri-Capsules with Isohelix SK-1 buccal swabs.

Introduction:

DNA buccal swabs provide a convenient, cost-effective alternative to invasive venopuncture for the collection of DNA for genotyping, diagnostics, 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 -20C 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 to 4 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.

Method:

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 12 months prior to analysis.

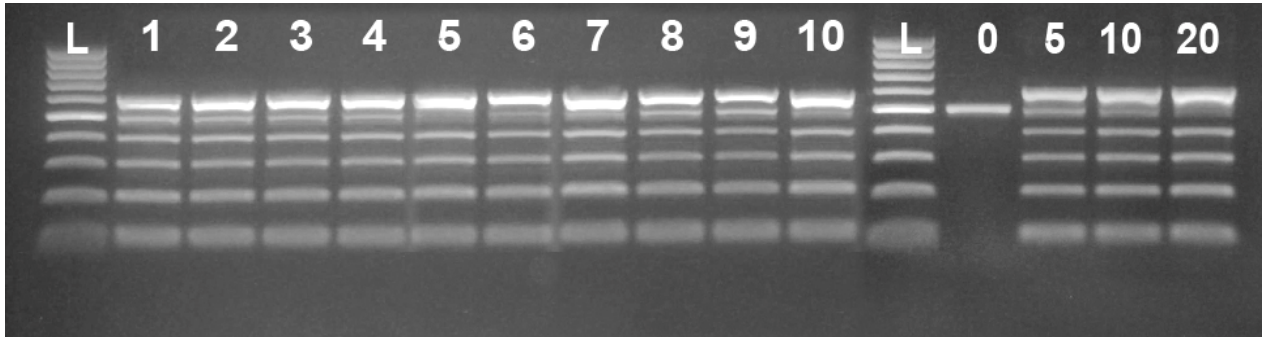
The DNA was isolated from the swabs using the Isohelix DDK DNA Isolation Kit and the quality of the isolated DNA was assessed using the Isohelix DQC-50 PCR Kit which is a multiplex PCR reaction specifically designed to check the quality and presence of human DNA in the isolated samples.

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 derived from an internal control, 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 from 12 month study:



| Lane | |
|--------|-----------------------------------|
| L | 100 bp ladder |
| 1 + 2 | Freshly taken swabs |
| 3 + 4 | Stored 1 month with Dri-Capsule |
| 5 + 6 | Stored 3 months with Dri-Capsule |
| 7 + 8 | Stored 6 months with Dri-Capsule |
| 9 + 10 | Stored 12 months with Dri-Capsule |
| 0 | Negative Control |
| 5 | 5ng Human DNA Control |
| 10 | 10ng Human DNA Control |
| 20 | 20ng Human DNA Control |

The results show that for all samples the DQC kit shows the DNA to be of good quality, intact and not degraded. High yields are seen on swabs taken at each of the time points confirming that there is no gradual reduction of stability or adverse affect on yield by storing the swabs with Dri-Capsules for up to 1 year.

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 1 year.

The study is ongoing and is expected to confirm an increased period of stability well in excess of the 12 months tested so far.



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.