

DNA stable for in excess of 3 and a half years with Isohelix DSK stabilising and lysis kit in ongoing studies.

Introduction:

Isohelix DNA buccal swabs offer new and improved yields which together with their non-invasive advantages position them as a viable and attractive alternative to blood venopuncture. Furthermore the swabs are a convenient and cost-effective collection device for DNA samples being used in many techniques including genotyping and population and paternity studies both in humans and veterinary situations.

DNA stability on swabs has been well documented and allows sufficient time for the swab to be returned to the laboratory within a short to medium period, without undue breakdown of the DNA. In many situations however, the swabs will be stored, often for extended periods of time, prior to the DNA being isolated for further studies. It is of the utmost importance that the DNA in these cases is stabilised long-term against the enzymatic activity which naturally breaks down the DNA rendering it unsuitable for downstream testing.

Methods such as air drying or freezing at -20°C have traditionally been used yet do not offer complete DNA Stability. The Isohelix stabilising and lysis kits have been designed to give proven long-term stability, whilst maximising the yield of DNA. The Isohelix Stabilising and Lysis Kit fully stabilises your DNA from enzymatic and microbial activity that occur naturally after buccal sampling. The kit is designed to store the samples at room temperature and shows no visible loss of stability for at least 2 years (ongoing tests to date). The DSK Kit also

fully lyses the cells, releasing additional DNA to further enhance yields, whilst the enzymatic inhibitor maintains the structural integrity of the DNA.

Method:

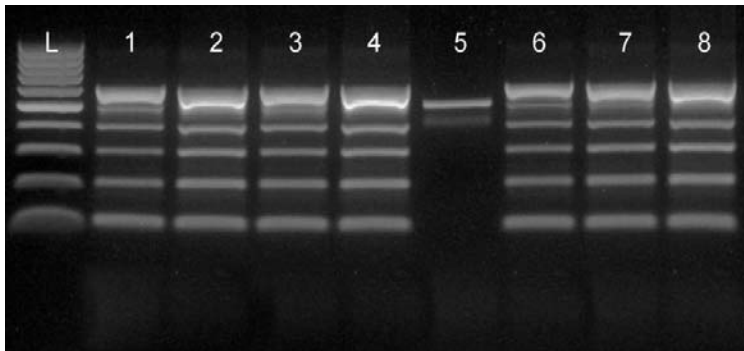
Multiple swabs were taken from individuals using Isohelix SK-1 swabs and treated identically by adding the LS and PK components of the DSK stabilising and lysis kit to the tubes containing the snapped off swab heads. Half of the swab samples were then processed using the Isohelix DDK isolation kit to isolate the DNA which was then stored at -20C . The remaining swabs were stored in the DSK stabilisation and lysis solution, on the bench at room temperature for up to 3 years and 9 months. After this period of time, the swab samples were processed identically using the DDK isolation kit to isolate the DNA.

The stability and quality of the isolated DNA was checked 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.

Results from 3.5+ year study:

DNA Stability study over 3 years and 9 months using Isohelix DSK Stabilising and Lysis Kit



L 100bp DNA Ladder
 1 1 month
 2 3 Years
 3 3 Years 9 Months
 4 3 Years 1 month
 5 TE Blank
 6 5ng Human DNA
 7 10ng Human DNA
 8 20ng Human DNA

For samples 1-4, buccal swabs were stabilised with DSK and the DNA isolated after the stated period using Isohelix DDK DNA Isolation Kit.

There is no observable difference between the DNA samples isolated from the fresh swab in 2010 and those isolated from the swabs that had been stored in the DSK stabilisation and lysis buffer at room temperature for up to 3 years and 9 months. All samples show the 6 bands generated by

the multiplex PCR reaction used in the DQC quality check kit, which indicate the DNA is of good quality and not denatured. In addition the intensities of the bands in comparison to the known standards indicate high yields of intact DNA have been isolated using the DDK DNA Isolation Kit.

Conclusions:

The DSK stabilisation and lysis kit provides a simple and reliable method to stabilise buccal swab DNA long term at room temperature. There has been no breakdown of the DNA after 3 years 9 months storage at room temperature in the stabilisation/lysis buffer. The testing is still on-going and with this current information we anticipate that any future long term stability testing is likely to

show consistently maintained DNA stability values. The stabilisation/lysis buffer fully lyses the buccal cells to make all the DNA available for subsequent isolation and at the same time, stabilises the DNA by protecting it from the enzymatic action of DNases and microbes naturally present in the samples after buccal swabbing.

Other Cell Projects Products

- **Isohelix DNA Buccal Swabs.**
High yields, blood alternative, reproducible, easy to use, different formats for various extraction methodologies. SK-1, SK-2, SK-3 and SK-4
- **Isohelix DNA Isolation and Handling kits**
DNA isolation kits optimised for high yields of intact DNA from buccal swabs. DDK-3/50
- **Isohelix DNA Silica Gel Capsules**
For use with SK-1 swab kits, air-dries swab in tube giving extended storage times without loss of stability. SGC-3/50
- **Isohelix DNA Quality Check Kit**
PCR kit to confirm quality of DNA prior to onward experimentation DQC-10/50
- **Isohelix Buccalyse Kit**
A quick and simple one tube method for extracting PCR-ready DNA from buccal swabs. BEK-25/SK1

Cell Projects specialise in buccal cell DNA sampling and isolation technologies and offer a range of Isohelix products together with full technical support in this area.

Further technical application notes are available to download from www.isohelix.com

For further information on any of our products please contact Cell Projects technical support at info@isohelix.com

Isohelix is a division of Cell Projects Ltd

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