

A rapid and simple one-step method to extract PCR-ready DNA from buccal swabs

Introduction:

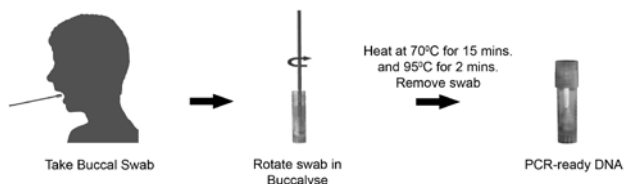
Buccal swab DNA sampling is commonly used for acquiring DNA samples for use in PCR-based diagnostic and clinical genotyping assays. The growth in popularity of these assays calls for quick and easy methods to produce PCR-ready DNA samples from buccal cell swabs. We describe here the use of a simple and rapid one-step/one-tube method to prepare PCR-ready DNA from Buccal swabs in under 10 minutes.

The Isohelix Buccalyse Kit has been specially formulated to produce high DNA yields from buccal swabs (2-4ug average total yield from an adult) and offers advantages of ease of handling, speed and ease of sample collection with rapid isolation of PCR-ready DNA and safety – no use of needles, blood handling or solvent based chemicals. The kit is also suitable for automation and HT formats as no centrifugation step is required.

The Buccalyse Kit contains reagents for 50 extractions, with the option to include 50 Isohelix buccal swabs. A sample kit with 3 extractions and swabs is also available.

Method:

Buccal swabs were taken from 5 individuals and the DNA extracted using Buccalyse according to the following protocol.



Add the swab to the tube containing 400ul Buccalyse.
 Rotate the swab in the Buccalyse solution to rehydrate and mix.
 Remove the swab from the tube or alternatively snap the swab stick off leaving the swab head in the tube.

Vortex briefly. Place the tube in a waterbath at 70°C for 15 minutes, vortex briefly. Reset the temperature of the waterbath to 95°C and once the temperature is achieved, leave the tube for 2 minutes. Remove the tube from the waterbath and vortex briefly. Remove the swab head if present. The DNA is now ready for use in PCR reactions or can be stored short term at 4C, or long term at 20C.

The Buccalyse extracted DNA can be used to make up to 40% of the total volume of the PCR reaction however for most PCR's 10% is ample (i.e. 2.5ul in a total reaction volume of 25ul).

Results:

The Buccalyse extracted DNA was tested for quality and competency for PCR in a multiplex PCR reaction using the Isohelix DQC quality check kit¹ (Cat No DQC-50). 2.5ul of each Buccalyse extracted DNA sample was used in a 25ul PCR reaction according to the protocol supplied with the DQC quality check kit, and 10ul of the PRC reaction was run on an agarose gel.

The DQC quality check kit is designed to produce 6 bands of 100, 200, 300, 400, 500 and 600bp in size. If all 6 fragments are observed the DNA is not denatured. The 500bp fragment is derived from an internal control and should always be present.

Quality check PCR on Buccalyse extracted DNA



L = 100bp Ladder
 S1 = sample 1
 S2 = sample 2
 S3 = sample 3
 S4 = sample 4
 S5 = sample 5
 B = Blank
 C1 = 2.5ng human DNA
 C2 = 5ng human DNA
 C3 = 10ng human DNA

The gel shows that good quality, intact DNA has been extracted from all 5 samples. By comparing the intensities of the bands against the human DNA standards we can estimate the concentration of the DNA to be between 5 and 10 ng/ul giving a total yield of 2 to 4 ug DNA on average from an adult.

Conclusions:

The Isohelix Buccalyse Kit allows rapid extraction of good quality PCR-ready DNA from buccal swabs which has been demonstrated to be of high biological quality in a multiplex PCR reaction.

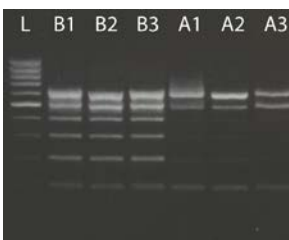
The yield of DNA from a single buccal swab using Buccalyse is generally around 2 to 4ug from an adult, which can give sufficient DNA for in excess of 100 PCR reactions, making the Buccalyse kit a quick and simple to use alternative to existing DNA isolation methods when extracting DNA for use in PCR reactions.

Comparison of Buccalyse with an alternative single tube method, tested using the Isohelix DQC PCR quality check kit is shown below.

2 buccal swabs were taken from 3 individuals and the DNA extracted using either Buccalyse (B1-3) or an alternative single tube method (A1-3).

2.5ul of the extracted DNA was used in a 25ul PCR reaction using the DQC quality check kit and 10ul of the PCR reaction run on a gel.

Comparison of Buccalyse to an alternative single tube kit



L = 100bp Ladder
 B1 = Buccalyse sample 1
 B2 = Buccalyse sample 2
 B3 = Buccalyse sample 3
 A1 = Alternative sample 1
 A2 = Alternative sample 2
 A3 = Alternative sample 3

In this test the Buccalyse Kit out-performs the alternative single-tube kit in terms of quality and quantity of the DNA as demonstrated by the DQC kit.

Notes:

¹ See Isohelix product application note DQC-50-01 for more details on the DQC quality check kit.

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

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, SK-4 and SK-5
- **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 Isolation and Handling kits**
 DNA stabilising kits for the stable storage of DNA at room temperature for long periods DSK-50
 DNA isolation kits optimised for high yields of intact DNA from buccal swabs. DDK-3/50
- **Isohelix DNA Quality Check Kit**
 PCR kit to confirm quality of DNA prior to onward

