BuccalQuick

Buccal Cell DNA extraction

User Manual V1.2

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Storage: Upon receipt, store the kit at 2-8°C.

Storage Information

Storage options	Shelf life
Kits stored at 2-8°C	6 months
Kits stored at –20°C	1 year

Limited Product Warranty

It is imperative that users strictly adhere to this manual. Failure to do so will void TrimGen's guarantee of this product. TrimGen Corporation makes no other warranties of any kind, expressed or implied, including without limitation, warranties of merchantability or fitness for a particular purpose.

This product is designed for "research use only, not use for diagnostic purpose". The purchaser must determine the suitability of the product for its particular use. No claim or representation is intended for use of this product to identify any specific organism or for a specific clinical use (diagnostic, prognostic, therapeutic, or blood banking).

Notice to Purchaser

The purchase of BuccalQuick™ products includes a limited, nonexclusive license to use the kit. This license does not grant rights to reproduce or modify the kit for resale, or to use the BuccalQuick™ kit to manufacture commercial products without written approval of TrimGen Corporation. No other license, expressed, implied or by estoppels is granted.

Product Safety and Liabilities

When working with the kit reagents, always wear a suitable lab coat, disposable gloves, and protective goggles. TrimGen Corporation shall not be liable for any direct, indirect, consequential or incidental damages arising out of the misuse, the results of use, or the inability to use this product.

Introduction

Buccal cells are a convenient source to collect genomic DNA for genetic analysis. This kit is designed to efficiently extract genomic DNA from a buccal swab or brush. The whole extraction process is performed in less than 10 minutes. No binding, wash, elute and centrifugation steps is required. The kit can directly extract the DNA from a form swab (TrimGen's Easy-Swab™) dried and stored in room temperature for 2 years after buccal cell collection.

FEATURES

Simple:	Single tube, few steps, no centrifugation.
High Yield:	DNA extracted from one buccal swab is sufficient to perform up to 100 PCR reactions*. ***** DNA yield may vary if using different types of swabs. The result is obtained from TrimGen's Easy-Swab™ (catalog number ES-100).
Time-Savings:	Less than 10 minutes extraction time.
Non-detergent:	Detergents will affect downstream application. The extraction buffer of BuccalQuick does not contain any type of detergent.
Application	Buccal Cell DNA extraction Culture Cell DNA extraction

Frequently Asked Questions

Q: I have a sample from a different type of swab, can I use the BuccalQuick™ to perform the DNA extraction?

A: Yes. You need to increase the soaking time in the Extraction Buffer. The swab type significantly affects the DNA release. In general, the cotton swab has a poor DNA release. TrimGen provides Easy-Swab™ (Cat No. ES-100) specially designed for buccal DNA collection. The Easy-Swab™ can release more than 90% of collected DNA. The DNA yield from one Easy-Swab™ is sufficient for up to 100 PCR applications.

Q: Do I need to adjust my PCR conditions?

A: No. The extraction buffer of BuccalQuick™ does not contain any type of detergent. The final extract can be directly used for PCR, it is not necessary to adjust the conditions of PCR amplification.

Q: How much of the final extract should be used for PCR?

A: PCR conditions vary by laboratory. The extraction buffer of BuccalQuick is optimized for most commercial PCR kits. It uses 1-2 μl for a 50 μl PCR reaction or 0.5-1 μl for 25-50 μl real-time PCR reactions. For home brew PCR, a titration of final extracts (0.5μl, 1μl, 2μl, 3μl, and 5μl) will help to find out the best condition for your PCR system.

Q. Can I use the kit to extract DNA from other cell samples

A: Yes. You can use the kit to extract the DNA from

Cultured cells
Isolated white blood cells

The cells need to be washed with PBS before the extraction.

Kit Contents

Component	BQ-50 50 extractions
BQ-Solution	18 ml
Enzyme Mix*	425 µl

*International customers are provided with lyophilized enzyme mix and dilution buffer. Use 500 µl buffer to solubilize the enzyme mix.

CAUTION!
The reagents contain hazardous chemicals. Wear suitable eye protection and gloves.

FIRST AID
In case of skin contact, flush with water. In case of ingestion or eye contact, seek medical attention.

Materials and Equipment Needed

2 ml screw-cap tubes

Laboratory incubator or water bath

Heat Block

Vortex Mixer

Procedure

Pre-heat an incubator (or water bath, or heat block) to 55°C and a heat block to 95°C.

1. Collect 2 ml screw-cap tube for each swab. Label the tube with sample ID and place the tube in a tube rack.

> ***Blank control:***
> Add an extra tube to Step 1. Label the tube as **BC** and process it as a sample without a swab. This BC tube serves as the **Blank Control** for $OD_{260/280}$ calibration.

2. Prepare **Extraction Buffer**

BQ-Solution	300 µl x _____ (total sample) = _____ µl
Enzyme Mix	7 µl x _____ (total sample) x = _____ µl

* For pipetting error.

Transfer above calculated volumes of both reagents to one tube and mix the contents by vortexing. This is the **Extraction Buffer**.

3. Transfer 300 µl of **Extraction Buffer** to each tube.

4. Place the swab head into the **Extraction Buffer** in the tube and twist the swab vigorously 8-10 times **(No swab for blank control tube)**. Before discarding the swab, try to remove all the liquid remaining in the swab head by pressing the swab head against the tube wall, then discard the swab.

> Alternative: Cut the swab head into the **Extraction Buffer**. Keep the swab head in the buffer during extraction.

5. Cap the tube and vortex for 10 seconds at high speed.

6. Incubate the tube at 55°C for 1-5min (a longer incubation will help to increase the yield of DNA).

7. Heat the tube at 95°C for 3 minutes.

8. The sample is ready for PCR.

DNA Storage

The extracted DNA can be **stored at -20°C**.

DNA Concentration Measurement:

Transfer 10 μl of the final extract to a new tube. Add 40 μl of water to make a 1:5 dilution. Use the diluted **Blank Control** to calibrate the OD260 and OD280 to zero, then measure the OD of samples.

The following equation can be used to estimate the DNA concentration.

DNA Conc. (ng/μl) = (62.9 x OD_{260} – 36 x OD_{280}) x dilution factor / 5

A convenient calculation form is available online:

http://trimgen.com/products/BQ-DNA-calculation.xls

DNA Quality
BuccalQuick™ is a homogeneous extraction method. The final extract includes DNA and proteins. A typical $OD_{260/280}$ ratio is *0.6-1.5*. The low OD ratio will not affect the PCR.

PCR Amplification

Regular PCR
Use 0.5-2μl of the final extract for a 25-50 μl PCR reaction.

Real-time PCR
Use 0.5-1μl* of the final extract for a 25-50μl real-time PCR reaction.

***Using excess final extract may negatively affect the real-time PCR reaction.**

The PCR result may vary depending on the PCR reagents used. For first time user, we recommend performing a titration test. Use 0.5μl, 1μl and 2μl of the final extract as a template for a 25-50μl PCR reaction to determine the best sample volume for your PCR.

An optimized PCR master mix is available at TrimGen (Master Mix, Cat No. GR048).

Troubleshooting Guide

Problem	Suggestions
OD ratio is below QC criteria, can I still use the extracted DNA for PCR?	Yes. BuccalQuick is a homogenous extraction method. The OD ratio is lower than column or bead extraction method. The low OD ratio will not affect the PCR amplification.
OD_{260} is too high	Use the Blank control (a reagent control) to calibrate the spectrophotometer and then measure the OD of sample.
DNA concentration calculated from the equation is low and subsequently PCR does not amplify properly	**Incomplete release of buccal DNA from the swab** The swab type and conditions significantly affect DNA release. In general, a cotton swab or swab with fungi growth gives poor DNA release. Soaking the swab for an increased period of time at 55 ^{o}C will help to release the DNA.
DNA concentration calculated from the equation is high but the PCR does not amplify properly.	**DNA concentration is too high** Dilute the final extract with water then perform the PCR. The extraction buffer is optimized for most commercial PCR kits. If you use a home brew PCR, the conditions may be different. To find out the best conditions for your PCR, a titration of the final extract (0.5μl, 1μl, 3μl and 5μl) will help you to find the best volume for PCR amplification.