

INTRODUCTION

DNA EasyXtract Solution provides an extremely efficient method for extracting PCR-ready genomic DNA from diverse samples. DNA EasyXtract Solution is used in a simplified single-tube reaction requiring only a heat treatment step without the need for centrifugation or further sample purification. Sample handling time is 4-10 minutes (protocol dependent) making it easier to process one to hundreds of samples in less than an hour without phenol, chloroform or other toxic organic solvents.

The provided protocols have been used to extract PCR-ready genomic DNA from human buccal cell swabs, mammalian tissue culture cells, *Escherichia coli* and other gram-negative bacterial cells, mouse tail snips, *Drosophila* and other insects, hair follicles, fingernails and feathers.

- Economical alternative to other available DNA Extraction Solutions
- Non-toxic
- Simple and fast protocols
- Standard, Rapid and Buccal Swab-based Protocols included

MATERIALS

Materials Supplied

DNA EasyXtract™ Solution		
Component	Volume	
	EXD26015 10 Extractions	EXD260150 100 Extractions
DNA EasyXtract™ Solution	5 ml	50 ml



Storage

DNA EasyXtract Solution may be stored at 4°C for up to one year, or at –20°C in a freezer without a defrost cycle. DNA EasyXtract Solution is stable for up to 30 freeze/thaw cycles. Optional: DNA EasyXtract Solution could be stored at –20°C in smaller-sized portions for user convenience.

SPECIFICATIONS

Unit Definition

One standard DNA extraction utilizes 0.5 ml of DNA EasyXtract Solution.

Functional Testing

The DNA EasyXtract Solution is function-tested by assaying for the production of a PCR product from human β -actin using a buccal cell DNA preparation as template.



For more information, consult the appropriate safety data sheet (SDS) at www.cellscript.com.

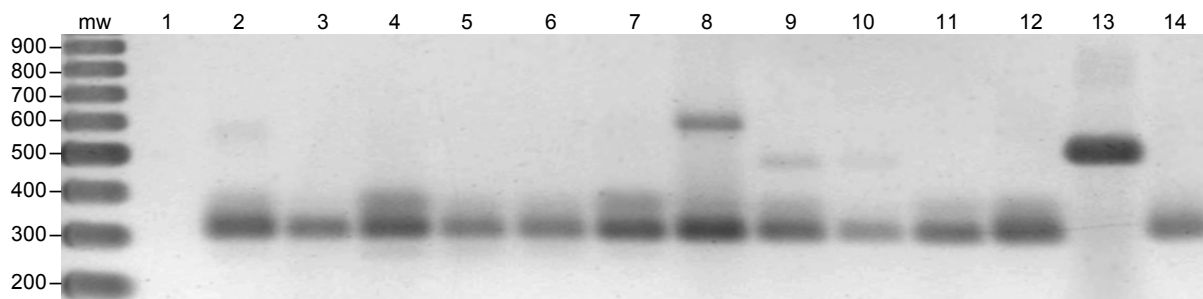
PROCEDURES

Three protocols are provided for use with DNA EasyXtract Solution. Choose the protocol which best fits your needs. The **Rapid Protocol** can be used for easy-to-lyse cells such as mammalian tissue culture cells, *Escherichia coli* and other gram-negative bacterial cells. The **Standard Protocol** should be used for samples which require longer lysis/digestion times such as mouse tail snips, *Drosophila* and other insects, hair follicles, finger nails and feathers. Extending the lysis/digestion incubation time may be necessary for even more difficult-to-lyse samples. Some user optimization of the lysis incubation time may be necessary for such substrates. The **Buccal Swab Protocol**, based on the Rapid Protocol, can be used for DNA extraction from human cheek swab samples.

A. Standard Protocol PCR-ready DNA Extraction

1. Dispense 0.5 ml of DNA EasyXtract Solution into a 1.5 ml microfuge tube per sample to be extracted.
2. Add cell/tissue sample to the tube.
Examples include:
 - 10⁴ mammalian tissue culture cells.
 - a 0.5-1 cm region of a single plucked human hair follicle.
 - a 0.5-1 cm section of a mouse tail snip, finely diced.
 - a 0.5-1 cm quill-end of a single plucked feather.
 - one single *E. coli* colony picked from a plate.
 - one single *Drosophila*.
3. Vortex mix for 15 seconds.
4. Incubate the tube at 65°C for 6 minutes.
5. Vortex mix for 15 seconds.
6. Incubate the tube at 98°C for 2 minutes.
7. Vortex mix for 15 seconds.
8. Quantitate DNA yield by fluorimetry to avoid an over estimation given by A₂₆₀ readings.
9. Store the DNA at -20°C, or at -70°C for long term storage.
10. Use ≤5 µl of the extracted DNA for PCR amplification (see Figure 1).

Figure 1. Standard Protocol DNA EasyXtract Reaction PCRs.

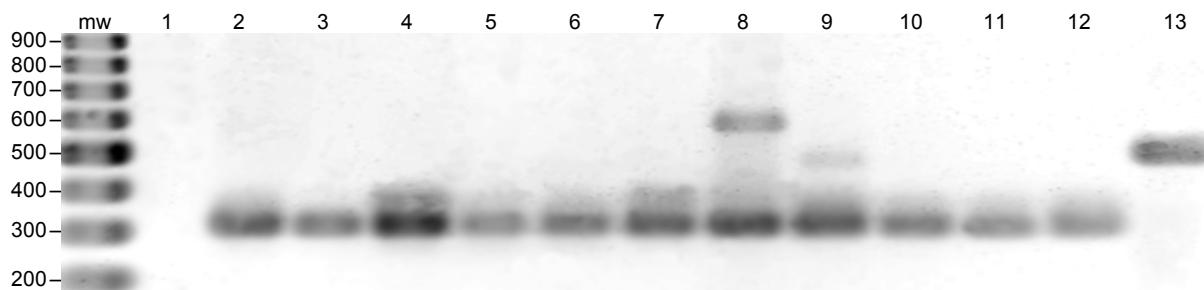


Human β -actin targeting PCR amplicons (except Lane 13), Lane 13: 16S rRNA targeting PCR amplicon

mw) size markers (bp)	4) human fingernail	8) mouse tail snip	12) <i>Drosophila</i>
1) no template control	5) HEK mammalian cells	9) avian claw	13) <i>E. coli</i>
2) human buccal swab	6) HeLa mammalian cells	10) avian feather	14) salami
3) human hair follicle	7) THP mammalian cells	11) waxworm	

B. Rapid Protocol PCR-ready DNA Extraction

1. Dispense 0.5 ml of DNA EasyXtract Solution into a 1.5 ml microfuge tube per sample to be extracted.
2. Add cell/tissue sample to the tube.
Examples include:
 - 10^4 mammalian tissue culture cells.
 - one single *E. coli* colony picked from a plate.
3. Vortex mix for 10 seconds.
4. Incubate the tube at 65°C for 1 minute.
5. Vortex mix for 15 seconds.
6. Incubate the tube at 98°C for 2 minutes.
7. Vortex mix for 15 seconds.
8. Quantitate DNA yield by fluorimetry to avoid an over estimation given by A_{260} readings.
9. Store the DNA at -20°C, or at -70°C for long term storage.
10. Use $\leq 5 \mu\text{l}$ of the extracted DNA for PCR amplification (see Figure 2).

Figure 2. Rapid Protocol DNA EasyXtract Reaction PCRs.

Human β -actin targeting PCR amplicons (except Lane 13), Lane 13: 16S rRNA targeting PCR amplicon.

mw) size markers (bp)	4) human fingernail	8) mouse tail snip	12) <i>Drosophila</i>
1) no template control	5) HEK mammalian cells	9) avian claw	13) <i>E. coli</i>
2) human buccal swab	6) HeLa mammalian cells	10) avian feather	
3) human hair follicle	7) THP mammalian cells	11) waxworm	

C. Buccal Swab Protocol PCR-ready DNA Extraction

This protocol requires user-provided, sterile, medical-grade buccal swabs for sample collection.

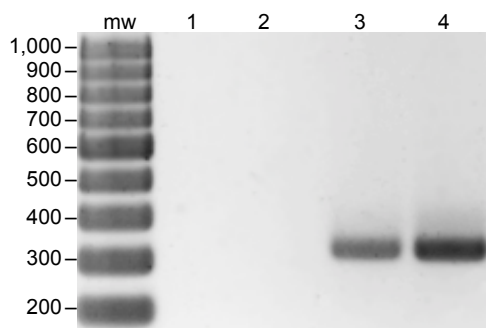
Sample Collection

1. Thoroughly rinse out the subject's mouth twice with water. Discard rinsate. We recommend that subjects abstain from drinking coffee before tissue collection. Alternatively, instruct subjects to gently brush the inside surface of both cheeks with a toothbrush (without toothpaste); follow with a thorough rinsing of the mouth with water. Discard rinsate.
2. Collect tissue by rolling the sample collection swab firmly on the inside of the cheek, approximately 20 times on each side, making certain to move the brush over the entire cheek. If storing or transporting the sample, air dry the swab for 10-15 minutes at room temperature. Store the dry swab in the original packaging at 22-37°C for up to one week before extracting the DNA. For longer storage, place the dry swabs in the original packaging at -20°C for up to 6 months. Yield is directly correlated with the starting amount of buccal cells. If yield is not a concern, use only one swab; if yield must be maximized, use a separate swab for each cheek surface, and if necessary, use a third swab, collecting tissue from both cheeks.

Sample DNA Extraction

3. Dispense 0.5 ml of DNA EasyXtract Solution into a 1.5 ml microfuge tube per sample to be extracted.
4. Place the swab end of the sample collection swab into the tube containing the DNA EasyXtract Solution and rotate the brush a minimum of 5 times. Press the brush against the side of the tube and rotate the brush while removing it from the tube to ensure most of the liquid remains in the tube.
5. Vortex mix for 10 seconds.
6. Incubate the tube at 65°C for 1 minute.
7. Vortex mix for 15 seconds.
8. Incubate the tube at 98°C for 2 minutes.
9. Vortex mix for 15 seconds.
10. Quantitate DNA yield by fluorimetry to avoid an over estimation given by A_{260} readings.
11. Store the DNA at -20°C, or at -70°C for long term storage.
12. Use ≤5 µl of the extracted DNA for PCR amplification (see Figure 3).

Figure 3. Buccal Swab Protocol EasyXtract Reaction PCRs.



Human β -actin targeting PCR amplicons.

mw) size markers (bp)

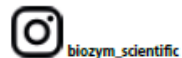
- 1) sample input: water (negative control)
- 2) sample input: DNA EasyXtract Solution-only (negative control)
- 3) sample input: 1 µl DNA EasyXtract Solution-treated buccal swab sample extract
- 4) sample input: 2 µl DNA EasyXtract Solution-treated buccal swab sample extract



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