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QuickExtract™ Bacterial DNA Extraction Kit

Cat. Nos. QEB0905T and QEB09050

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1. Introduction

The QuickExtract™ Bacterial DNA Extraction Kit is used to extract DNA from Gram-positive and Gram-negative bacteria. The kit contains Ready-Lyse™ Lysozyme Solution, with over 200 times the specific activity of hen egg lysozyme, and QuickExtract Solution formulated for bacterial DNA extractions.

The protocol is simple: add QuickExtract Solution and Ready-Lyse Lysozyme Solution to a bacterial pellet or sample containing bacteria, and incubate at room temperature for 15 minutes. To kill any remaining viable bacteria, the samples optionally may be heated to 80°C for 2 minutes.

The DNA obtained can be used for PCR, pulse-field gel electrophoresis (PFGE), restriction digestion, or optical mapping.

2. Kit Contents

Cat. #		Quantity
QuickExtract™ Bacterial DNA Extraction Kit Contents		
The QuickExtract Bacterial DNA Extraction Kit is available in 5-ml and 50-ml sizes, sufficient to perform 50 and 500 standard bacterial DNA extractions respectively.		
5 ml (50 Extractions)		
QuickExtract™ Bacterial DNA Extraction Solution		
QEB0905T		5 ml
Ready-Lyse™ Lysozyme Solution	**50 µl**	
50 ml (500 Extractions)		
QuickExtract™ Bacterial DNA Extraction Solution		
QEB09050		50 ml
Ready-Lyse™ Lysozyme Solution		500 µl

3. Product Specifications

Storage: Store the QuickExtract Bacterial DNA Extraction Kit at –20°C in a freezer without a defrost cycle.

Storage Buffer: Ready-Lyse Lysozyme Solution is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, and 0.1% Triton® X-100

Quality Control: The QuickExtract Bacterial DNA Extraction Kit is function-tested by purifying DNA from *Brevibacterium linens*, and assaying for the production of an ~300-bp PCR product from the *rpoB* gene encoding an RNA polymerase beta subunit.

4. Related Products

The following products are also available:

- FailSafe™ PCR PreMix Selection Kit
- MasterPure™ Gram Positive DNA Purification Kit
- FailSafe™ PCR System

5. Notes on Applications for the QuickExtract Bacterial DNA Extraction Kit

1. If the kit is used for general bacterial lysis and DNA extraction, the time required for lysis and clearing of the solution will vary with the type of microorganism. The kit will function for many Gram-positive and Gram-negative bacteria, but the time required for lysis will vary from 15 minutes to several hours at room temperature. It is imperative to test a range of incubation times at room temperature for lysis of each organism.
2. For PFGE or optical mapping samples, when very long genomic DNA (gDNA) is a prerequisite, the quantity of cells lysed with QuickExtract Bacteria DNA Extraction Solution may need to be titrated. The process of lysis and DNA extraction will be more rapid and more complete with fewer cells. The standard protocol is designed for approximately 10^8 bacteria or about 0.1 ml of a saturated bacterial culture. Please note that the DNA obtained with this kit can exceed 300 kb in length. Once DNA is obtained, it will need to be diluted for downstream applications.

6. QuickExtract Bacterial DNA Extraction Kit Protocol

1. Centrifuge ~10^8 bacteria at 1,700 x g (5,000 rpm) in a microcentrifuge for 3 minutes to pellet the cells.
2. Wash the bacterial cell pellet once with 0.5 ml of sterile water, then recentrifuge at 1,700 x g (5,000 rpm) for 3 minutes.
3. Carefully remove and discard the supernatant. Add 100 µl of QuickExtract Bacterial DNA Extraction Solution to the cell pellet.
4. Add 1 µl of Ready-Lyse Lysozyme Solution to each tube and mix gently by inversion. Make certain that both the bacteria and the Ready-Lyse Lysozyme are dispersed in solution, but avoid actions that could cause shearing of the DNA if long gDNA is required.
5. Incubate the suspension at room temperature for 15 minutes. If the solution is not clearing, wait an additional hour at room temperature. Observe the lysis periodically; digestion can be extended to several hours if necessary. **Optional:** If it is important to kill any remaining viable bacteria, the sample may be heated at 80°C for 2 minutes.
6. The DNA is now ready for PCR, restriction endonuclease digestion, PFGE, or optical mapping.

Note: *If the DNA is to be used for PCR, use at full strength or dilute in TE Buffer (10 mM Tris-HCl pH=7.5, 1 mM EDTA). For restriction digests, DNA can be used at full strength or diluted before adding the appropriate 10X restriction endonuclease buffer and enzyme.*

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