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Direct DNA Extraction Kit (Bacteria) - 50 Preps Product Insert

Product# 61500

Principle of the Test

Norgen's Direct DNA Extraction Kit (Bacteria) contains Buffer DL and pre-filled bead tubes for the extraction of DNA from enriched Gram negative and positive bacteria from food and culture samples. The kit first allows for the extraction of DNA directly from bacteria using mechanical homogenization with Buffer DL. The cell debris is then removed by a short centrifugation and the clean supernatant is ready for use in PCR reactions. Buffer DL is designed to stabilize extracted DNA and inactivate PCR inhibitors to provide a rapid and economic solution for high throughput pathogen detection, particularly for Gram-positive bacteria such as *Listeria spp*. Norgen's Direct DNA Extraction Kit (Bacteria) is compatible with sensitive detection applications including end-point PCR and real-time PCR.

Kit Components:

Component	Product #61500 (50 Preps)
Buffer DL	2 x 25 mL
Bead Tubes	50
Product Insert	1

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge (>20,000 x g)
- 1.5 mL or 2 mL Nuclease-free microcentrifuge tubes
- Flat bed vortex or bead beater equipment (e.g. MP Biomedicals' FastPrep®-24 Instrument)
- Stomacher, blender or equivalent
- Incubators capable of maintaining 30ºC, 36ºC or variable temperature setting

Storage Conditions

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers

General Precautions and Disclaimers

Enriched cell cultures from food samples or bodily fluid are considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken. Norgen's Direct DNA Extraction Kit (Bacteria) is designed for research purposes only.

Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Safety Information

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at www.norgenbiotek.com.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Protocol

Important Notes Prior to Beginning Protocol:

For the enrichment procedure please follow a guideline complying with the requirements of local official standard procedure.

1. Sample Preparation and Enrichment (example)

a. To ensure a representative analytical unit, agitate liquids or free flowing materials until the contents are homogeneous. If the sample unit is a solid, obtain the analytical unit by taking a portion from several locations within the sample unit.

b. Add 25 g or mL of the food (the analytical unit) to 225 mL of the appropriate enrichment media in a blender jar or stomacher bag. For composite samples, analytical units may be combined up to 125 g or mL. If alternate analytical units are required, maintain a ratio of 1 part sample material to 9 parts enrichment media. Blend, stomach or vortex as required for thorough mixing. Proceed to step 1 d.

c. For environmental monitoring, collect environmental surface samples with a sponge or swab. After collecting sample, add sponge or swab to 100 mL or 10 mL respectively of the appropriate enrichment media. Masticate sponge and media to mix well. Proceed to step 1 d.

d. Incubate the mixed sample according to the recommended temperature and time for the bacteria of interest.

2. Lysate Preparation

a. Transfer 1 mL enriched cell culture (from previous sample preparation and enrichment section) to a DNase-free 1.7 mL microcentrifuge tube (not provided) and centrifuge at 20,000 x g (~14,000 RPM) for 1 minute. Completely remove the supernatant including fat with a pipette.

Note: If any cell culture media or food derived fat still is remaining, resuspend the pellet with 400 µL of Buffer DL and centrifuge at 20,000 x g (~14,000 RPM) for 1 minute. Completely remove the supernatant with a pipette.

b. Add 400 µL of Buffer DL to the cell pellet and resuspend by vortexing or gently pipetting.
c. Transfer the cell suspension from 2b to a provided Bead Tube.
d. Secure tube horizontally on a flat-bed vortex pad with tape, or secure the tube in any commercially available bead beater equipment (e.g. MP Biomedicals' FastPrep®-24 Instrument). Vortex for 10 minutes using a flat-bed vortexer at maximum speed or 1 minute at 4M/S using a FastPrep®-24 instrument.
e. Spin the lysate at 20,000 x g (~14,000 RPM) for 3 minutes to pellet any cell debris.
f. Transfer 100 µL of the supernatant to another nuclease-free microcentrifuge tube (not provided).

Note: Ensure that only the clear supernatant is transferred, avoiding any of the debris. If necessary, repeat Steps **1e and 1f** if visible precipitates are still present in the 100 µL supernatant.

g. For analysis, 2 µL supernatant can be used directly in a PCR including real-time PCR.

Note: The PCR input volume can be optimized based on the total PCR reaction or routine lab protocol.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
DNA does not perform well in downstream applications	Too much DNA template	Reduce input volume from 1 mL to 0.5 mL or dilute the sample from step 2e in a ratio of 1:10 in Buffer DL.
	PCR inhibitors from cell culture media or food	If food related PCR inhibitors or cell culture media interferes with the PCR, the cell pellet can be washed with 400 µL of Buffer DL as described in step 2a.
	PCR reaction condition is needed to be optimized	Take steps to optimize the PCR conditions being used, including varying the amount of DNA template, changing the source of *Taq* polymerase, looking into the primer design and adjusting the annealing condition.

Related Products	Product #
TaqMan 2x PCR Master Mix & TaqMan 2x RT-PCR Master Mix	28340, 28341
PCR Purification Kit	14400

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Direct DNA Extraction Kit (Bacteria) or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362 or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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