

User Manual

BacterialXpress™ Nucleic Acid Extraction Kit

50 Extractions

3096**FOR RESEARCH USE ONLY****Not for use in diagnostic procedures. Not for Human or Animal Consumption.**

Product Overview

The Light Diagnostics™ BacterialXpress™ Nucleic Acid Extraction kit is intended for the purification of bacterial DNA from plasma, serum, cerebral spinal fluid, amniotic fluid, tissue, bone marrow, and cell culture. The BacterialXpress™ kit provides a rapid method for the extraction and purification of amplification-competent nucleic acids; however, the kit does not separate DNA from RNA. The BacterialXpress™ Nucleic Acid Extraction Kit can be used for the extraction and purification of a broad range of bacterial DNA from a variety of sources. However, performance cannot be guaranteed for all bacteria from all sources.

FOR LABORATORY USE ONLY.

Materials Provided

Bacterial DNA Extraction reagent (5539)-1 bottle, 11.0 mL.

Materials Required (Not supplied)

- Nuclease-free Water (3098)
- Isopropanol
- 70% (vol/vol) Ethanol
- Nuclease-free polypropylene centrifuge tubes (1.5 mL)
- Microcentrifuge

Warnings and Precautions

Light Diagnostics™ BacterialXpress™ nucleic acid extraction reagent removes endogenous nucleases. Certified nuclease-free plasticware should be used whenever possible and work surfaces should be cleaned frequently with a diluted bleach solution to reduce the possibility for nuclease contamination.

Proper aseptic technique should be used when working with potentially infectious material. The BacterialXpress™ reagent contains a chaotropic salt, which is an irritant and is not compatible with disinfecting agents that contain bleach.

Dispose chaotropic salt containing solutions and plasticware according to your institutions waste-disposal guidelines.

The BacterialXpress™ reagent contains chaotropic salts, which may irritate the skin and damage clothing. As with any extraction procedure, use gloves, a laboratory coat and safety goggles. Avoid breathing vapors. In case of contact, immediately flush eyes or skin with a large amount of saline or water for at least 15 minutes.

Storage and Stability

BacterialXpress™ references a 2-8 °C storage temperature, however, the product can also be stored at room temperature upon arrival. Reagent will crystallize upon storage at 2-8 °C and should be re-dissolved with gentle warming at 37 °C before use.

Tissues

BacterialXpress™ can also be used to extract nucleic acids from tissues. Prior to extraction from solid tissue, the tissue should be homogenized in PBS and solid particles should be allowed to settle for 1 minute at 4 °C. 50 µL of supernatant can then be extracted.

Protocol

Extraction Procedure

DO NOT ALLOW THE EXTRACTION REAGENT TO COME IN CONTACT WITH BLEACH.

1. Transfer 200 µL of BacterialXpress™ nucleic acid extraction reagent to a nuclease-free microfuge tube (1.5 mL).
2. Add 50 µL of sample to each microfuge tube containing BacterialXpress™ extraction reagent, vortex for 10 seconds and incubate for 5 minutes at room temperature.
3. Add 250 µL of isopropyl alcohol to each tube, vortex for 10 seconds and centrifuge at 16,000 x g for 10 minutes at room temperature.
4. Discard supernatant and wash the pellet by adding 400 µL of 70% ethanol, vortex for 10 seconds and centrifuge at 16,000 x g for 10 minutes at room temperature.
5. Remove the supernatant and air dry.

NOTE: Incomplete drying may result in inhibition of subsequent amplification reactions. Brief drying under vacuum may be used to ensure complete removal of ethanol.

6. Resuspend the pellet in 50 µL of nuclease-free water.

RNA Removal

Use the following suggested protocol to remove RNA by using RNase.

For purification of DNA only:

Add 1.0 µL (10 units) of DNase-Free RNase (Promega, M4261) and 5 µL of 10X digestion buffer (100 mM Tris-HCl [pH 7.5], 50 mM EDTA, 2 M Sodium Acetate) to the 50 µL extracted nucleic acid preparation. Incubate at 37 °C for 30 minutes. Re-extract the sample with BacterialXpress™.

Troubleshooting

- The BacterialXpress™ reagent contains glycogen, which may affect the efficiency of amplification methods. Spiking experiments should be performed with higher concentrations of extracted nucleic acids to identify any deleterious effects.
- Nucleic acid pellets extracted with the BacterialXpress™ reagent, which contains glycogen, may appear wet after air drying. A vacuum centrifuge may be used to dry off residual ethanol; however, excessive drying with heat is unnecessary and may render the pellet refractory to re-suspension in water.

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Document Template 00007539FM, Ver 13.0

3096 Ver 6.0, Rev 27JAN2026, AV

