

Purification of genomic/viral DNA and RNA from stool samples using the VERSANT kPCR Sample Prep System and the VERSANT Sample Preparation 1.0 Reagent Kit

This protocol is designed for isolation of human or viral DNA and RNA from stool samples using the VERSANT® kPCR Sample Prep system and the VERSANT Sample Preparation 1.0 Reagent Kit.

Introduction

The VERSANT Sample Preparation 1.0 Reagent Kit is a reagent kit for the isolation of RNA and DNA. The kit can be used for extraction of nucleic acids in conjunction with the VERSANT kPCR Sample Prep system. The VERSANT Sample Preparation 1.0 Reagent Kit includes a lysis reagent which lyses the cells and denatures nucleases while leaving RNA and DNA intact. The released nucleic acids are captured on magnetic beads. Three different wash buffers are used to remove all proteins, nucleases and other contaminants, and then purified nucleic acids are eluted.

Notes

Please refer to the following instructional materials for the VERSANT kPCR Sample Prep System before beginning this protocol:

- *VERSANT kPCR Molecular System SP Application Guide (10379670 Rev. A, 2009-06)*
- *VERSANT Stand-alone Sample Prep (kPCR) Operator Checklist (NA0896 Rev. 0)*

Materials provided in the VERSANT Sample Preparation 1.0 Reagent Kit include:

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|---|---|
| Box 1 <ul style="list-style-type: none">• Magnetic beads• Lysis Buffer• Wash Buffer 1• Wash Buffer 2• Wash Buffer 3• Elution Buffer | Box 2 <ul style="list-style-type: none">• Proteinase K |
|---|---|

Additional materials required by this protocol but not included in the VERSANT Sample Preparation 1.0 Reagent Kit:

- VERSANT kPCR Sample Prep Module
- Nuclease-free water
- 1.5 mL Sarstedt microtubes (Sarstedt, PN 72.692.005) or equivalent for pre-processing of stool sample (e.g., dilution and incubation steps)
- 12 x 75 mm, 5-mL sterile polypropylene round bottom sample tubes (Falcon, PN 352063) or equivalent, for placing the diluted stool sample on the VERSANT kPCR Sample Prep module
- Heating block or equivalent
- Vortex mixer
- Quick-spin, bench-top microcentrifuge

Supplementary Protocol

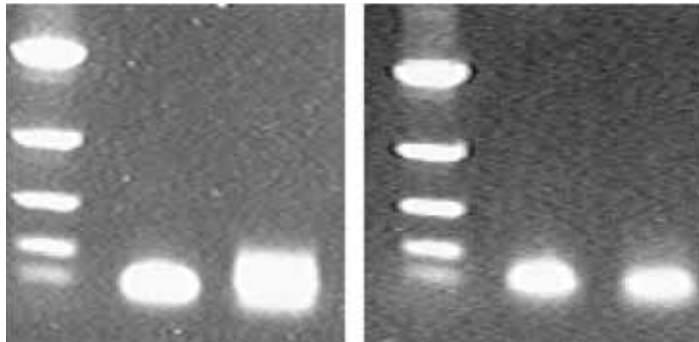
Stool Sample

Procedure

1. Follow the instructions as detailed in the *VERSANT Stand-alone Sample Prep (kPCR) Operator Checklist*. The following sections should be completed prior to preparation of the stool samples:
 - a. Preparing for a Run
 - b. Thaw Samples (if frozen)
 - c. Daily Maintenance
 - d. Preparing the Sample Preparation Module
 - e. Loading Plate and Tip Carriers
 - f. Preparing the VERSANT Sample Preparation Reagents
2. Dilute stool samples by transferring a small amount into a microtube and diluting with nuclease-free water to make a 20% to 40% solution as follows:
 - a. Pipette 600 μ L of nuclease-free water into 2-mL Sarstedt microtube, or equivalent.
 - b. Use a spatula (or equivalent) to transfer between 0.07 g to 0.240 g of stool to the microtube containing 600 μ L of nuclease-free water.
3. Vortex for five (5) seconds to thoroughly mix.
4. Incubate the diluted stool samples at 80°C for 30 minutes.
5. Centrifuge the diluted stool samples in microtubes at > 8000 rpm for 1 minute.
6. Transfer 300 μ L of supernatant into a properly labeled 5 mL tube.
7. Place the tube containing the diluted stool sample onto the sample carriers and proceed with the *VERSANT Stand-alone Sample Prep (kPCR) Operator Checklist* instructions, continuing with the set-up and run instructions as detailed in the following sections:
 - a. Preparing the Samples
 - b. Starting a Sample Preparation Run
 - c. Start the run using Protocol # 3 (100 μ L input volume and 100 μ L eluate volume)

Figure 1: Results* for PCR amplification of DNA and RNA targets extracted from stool using this protocol. Samples were prepared by spiking targets into stool samples at concentrations ranging from 100,000 to 1,000,000 copies/mL. (CMV is Cytomegalovirus, CT is Chlamydia, HIV is Human Immunodeficiency Virus, MTHFR is a human gene.)

Figure 1. Gel of PCR Amplification Products



Lanes and PCR Products from stool

1. Molecular Weight Standard
2. CMV PCR product
3. HIV RTPCR product

Lanes and PCR Products from stool

1. Molecular Weight Standard
2. CT product
3. MTHFR PCR product

Gel shows amplicon products of expected size were generated by CMV, CT, HIV and MTHFR PCR assays from stool samples spiked with these analytes and extracted using this supplementary protocol.

It is the user's responsibility to validate the performance of the VERSANT kPCR Sample Prep System and VERSANT Sample Preparation 1.0 Reagent Kit for any particular application, since their performance characteristics have not been validated for any specific use (research, diagnostic, prognostic, or therapeutic). This procedure may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.

*Internal data generated by Siemens Healthcare Diagnostics, Research and Development, Berkeley, CA, USA

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