

Purification of viral DNA and RNA from nasopharyngeal swabs using the VERSANT kPCR Sample Prep System and the VERSANT Sample Preparation 1.0 Reagent Kit

This protocol has been adapted by customers for use with the VERSANT® kPCR Sample Prep System and the VERSANT Sample Preparation 1.0 Reagent Kit. This protocol has not been thoroughly tested and optimized by Siemens.

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:

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

- VERSANT kPCR Sample Prep Module
- 1.5 mL Sarstedt microtubes (Sarstedt, PN 72.692.005) or equivalent
- 12 x 75 mm, 5-mL sterile polypropylene round bottom sample tubes (Falcon, PN 352063) or equivalent, for placing the sample on the VERSANT kPCR Sample Prep module
- Vortex mixer
- Quick-spin, bench-top microcentrifuge

User Developed Protocol

Nasopharyngeal Swab

Procedure

1. Collect nasopharyngeal swab specimens from symptomatic patients using a polyester, rayon, or nylon tipped swab. Place swab into a commercially available transport medium.

Per CDC guidelines¹, it is recommended that the following steps be carried out under a biological safety cabinet:

- a. Put 800 µl lysis buffer into each microtube.
 - b. Open the swab collection device and transfer the swab into the lysis buffer.
 - c. Repeat this procedure for all swab samples.
 - d. Next, remove the swabs from each tube, starting with the first sample so that only transport media remains in the microtube.
 - e. Discard the swabs.
2. Switch on VERSANT kPCR Sample Prep module and perform the recommended daily maintenance. Load the system with all required consumables including tips, 2 deep well plates and one 96-well PCR plate.
 3. Pour the VERSANT Sample Preparation Reagents into the reagent troughs and place them on the VERSANT Sample Prep module.
 4. Vortex the samples gently and then centrifuge them. Transfer 675 µL of sample into a properly labeled 5-mL sterile polypropylene round bottom sample tube.
 5. Load the samples onto the sample carrier. When placing the samples in the sample carrier, carefully remove the tube caps.
 6. Place the sample carriers on the auto load tray of the VERSANT Sample Prep module.
 7. The VERSANT kPCR Sample Prep System is fully automated from this step onward. At the Main Menu Screen: Select Protocol #1 (500 µL of liquid sample for extraction / delivers 100 µL eluate) or Protocol #4 (500 µL of liquid sample for extraction / delivers 50 µL eluate).
 8. Start run.
 9. Run time: Approximately 1½ – 3 hours for extractions of 1 to 96 samples (depending on batch size).
 10. At the conclusion of the run, remove the 96-well PCR plate containing the sample extracts. Sample extracts are now ready for amplification detection step.

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.

1. http://www.cdc.gov/h1n1flu/guidelines_labworkers.htm

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