User-developed protocol

User-Developed Protocol:

For purification of Norovirus RNA from human stool samples using the BioSprint® 96 workstation

This protocol has been adapted by customers and is for purification of Norovirus nucleic acid from human stool samples using the BioSprint 96 DNA Blood Kit in combination with the BioSprint 96 workstation. **The procedure has not been thoroughly tested and optimized by QIAGEN.**

Introduction

The BioSprint 96 workstation uses MagAttract® magnetic-particle technology for rapid purification of nucleic acids. MagAttract technology combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles and enables purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities. The purified nucleic acids are ready for direct use in downstream applications, such as amplification or other enzymatic reactions.

This procedure has been adapted by customers from the BioSprint 96 DNA Blood protocol. The BioSprint 96 DNA Blood Kit can be used to purify nucleic acids from viruses. However, kit performance is not guaranteed for each virus species and must to be validated by the user. **This protocol is intended for molecular biology applications. This protocol is neither intended for the diagnosis, prevention, or treatment of a disease, nor has it been validated for such use either alone or in combination with other products.**

IMPORTANT: Read the *BioSprint 96 DNA Blood Handbook*, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure. Ensure that you are familiar with the proper operation of the BioSprint 96 and with the *BioSprint 96 User Manual*.

Equipment and reagents to be supplied by the user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN® kit and kit component.

- BioSprint 96 workstation, cat. no. 9000852
- Magnetic head for use with large 96-rod covers (supplied with the BioSprint 96)
- Large 96-Rod Covers (16), cat. no. 1031668
- 96-Well Microplates MP (20), cat. no. 1031656
- S-Blocks (24), cat. no. 19585
- "BS96 vNA Noro Lysis" and "BS96 vNA Noro Isol" protocols, available from QIAGEN Technical Services or your local distributor
- BioSprint 96 DNA Blood Kit, cat. no. 940054 or 940057

- Buffer EB (250ml), cat. no. 19086
- Carrier RNA (12 x 1350 µg), cat. no. 1017647
- Ethanol (96–100%)*
- Isopropanol
- Stepper pipettor and appropriate disposable pipet tips (20–1000 µl) (e.g., Finnpipette® Stepper and Finntip® Stepper available from Thermo Electron, www.thermo.com)†
- Tubes for storing purified nucleic acids
- Soft cloth or tissue and 70% ethanol or other disinfectant to clean the worktable

Sample preparation

Resuspend human stool to obtain a 10% suspension. Centrifuge in a microcentrifuge tube for 1 minute at 14,000 x *g*. Use 200 µl of supernatant as sample input.

Preparing reagents

Dissolving carrier RNA and adding to Buffer AL

Add 1350 µl Buffer EB to a tube containing 1350 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly, dispense into conveniently sized aliquots, and store at –20ºC. Do not freeze–thaw the aliquots more than 3 times. Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer EB before adding to Buffer AL.

Add carrier RNA solution to Buffer AL. The purification procedure is optimized so that 2 µg carrier RNA is added per sample. For 96 samples, add 200 µl carrier-RNA solution to 20.8 ml Buffer AL. Mix gently by inverting the tube 10 times. To avoid foaming, do not vortex.

Reconstituting QIAGEN Protease

Add 5.5 ml Protease Solvent to a vial of lyophilized QIAGEN Protease to give a concentration of 1x protease solution. Label the vial accordingly. Mix carefully to avoid foaming. Make sure that the QIAGEN Protease is completely dissolved. Store the reconstituted QIAGEN Protease at 2–8ºC. We recommend freezing aliquots at –20ºC.

Preparing Buffer AW1

Add 35 ml ethanol (96–100%) to a bottle containing 27 ml Buffer AW1 concentrate, as described on the bottle. Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW1 is stable for 1 year when stored at room temperature (15–25ºC).

Note: Always mix reconstituted Buffer AW1 by shaking before starting a purification procedure.

* Do not use denatured alcohol, which contains other substances such as methanol or methyethylketone.
† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

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Preparing Buffer AW2

Add 160 ml ethanol (96–100%) to a bottle containing 66 ml Buffer AW2 concentrate, as described on the bottle. Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW2 is stable for 1 year when stored at room temperature (15–25ºC).

Note: Always mix reconstituted Buffer AW2 by shaking before starting a purification procedure.

Important points before starting

- Ensure that MagAttract Suspension G is fully resuspended. Vortex for at least 3 minutes before the first use. Vortex for 1 minute before each subsequent use.
- Ensure that QIAGEN Protease, Buffer AW1, and Buffer AW2 have been prepared according to the instructions on pages 2–3.
- 96-rod covers are supplied either as packets of two, or as packets of one inserted into an S-Block. If using a new packet of two, store the second 96-rod cover on another plate. It is important that the 96-rod cover does not become bent.

Procedure:

1. **Pipet 20 µl QIAGEN Protease to the bottom of an S-Block well. Add 200 µl sample to the QIAGEN Protease.**

   Note: Record position of the wells into which the samples are loaded.

2. **Add 200 µl Buffer AL containing carrier RNA.**

   To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution.

   Note: Do not add QIAGEN Protease directly to Buffer AL.

3. **Switch on the BioSprint 96 at the power switch.**
4. **Slide open the front door of the protective cover.**
5. **Select the "BS96 vNA Noro Lysis" protocol using the ⮝ and ⮟ keys on the BioSprint 96 workstation. Press "Start" to start the protocol run.**
6. **Load slot 2 of the worktable with the 96-rod cover (see table below) as instructed by the message appearing on the display, and press "Start".**

   The worktable rotates and a new message appears.

7. **Load slot 1 with the sample plate as instructed, and press "Start" again.**

Slot	Message when loading	Plate/block	To add	Volume per well
2	Load Rod Cover	96-well microplate MP	Large 96-rod cover	—
1	Load Sample Plate	96-well microplate MP	Sample	420 µl

Note: Each slot is labeled with a number. Load each 96-well plate or S-Block so that well A1 is aligned with the slot label (i.e., well A1 faces inward).

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8. **Check that the protective cover is correctly installed: it should fit exactly into the body of the BioSprint 96. Slide the door shut to protect samples from contamination.**

   Warning: Avoid contact with moving parts during operation of the BioSprint 96. See the *BioSprint 96 User Manual* for safety information.

   Note: See "Safety Information" in the *BioSprint 96 DNA Blood Handbook*.

9. **Press "Start" to start sample lysis.**

10. **Prepare three S-Blocks and two 96-well microplates according to the table below.**

    The S-Blocks and microplates are loaded onto the worktable in step 13 of the procedure.

    In each plate or block, the number of buffer-filled wells should match the number of samples to be processed (e.g., if processing 48 samples, fill 48 wells per plate or block). Ensure that buffers are added to the same positions in each plate or block (e.g., if processing 48 samples, fill wells A1–H1 to A6–H6 of each plate or block).

Slot	Message when loading	Plate/block	To add	Volume per well (µl)
6	Load Rod Cover	96-well microplate MP	Large 96-rod cover	—
5	Load Elution	96-well microplate MP	Buffer EB	100
4	Load Wash 3	S-Block	Buffer AW2	500
3	Load Wash 2	S-Block	Buffer AW2	500
2	Load Wash 1	S-Block	Buffer AW1	750
1	Load Lysate	S-Block	Lysate*	650

** Added at steps 2, 3, and 5; volume of lysate includes QIAGEN Protease, Buffer AL, MagAttract Suspension G, and isopropanol.*

11. **After the lysis protocol is completed, remove the plate containing the lysate.**

12. **Add 30 µl MagAttract Suspension G and 200 µl isopropanol to each sample in the S-Block, and return the S-Block to the working table.**

    Note: Ensure that the MagAttract Suspension G is fully resuspended before adding. Vortex for 3 min before using for the first time. Vortex for 1 min before each subsequent use.

13. **Select the "BS96 vNA Noro Isol" protocol using the ⮝ and ⮟ keys on the BioSprint 96 workstation. Press "Start" to start the protocol run.**

14. **Load slot 6 of the worktable with the 96-rod cover (see table on page 3) as instructed by the message on the display, and press "Start".**

    The worktable rotates and a new message appears.

15. **Load slot 5 with the elution plate as instructed. Continue to load the worktable by pressing "Start" and following instructions until all slots are loaded.**

    Note: Each slot is labeled with a number. Load each 96-well plate or S-Block so that well A1 is aligned with the slot label (i.e., well A1 faces inward).

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16. **Check that the protective cover is correctly installed: it should fit exactly into the body of the BioSprint 96. Slide the door shut to protect samples from contamination.**

    Warning: Avoid contact with moving parts during operation of the BioSprint 96. See the *BioSprint 96 User Manual* for safety information.

    Note: See “Safety Information” in the *BioSprint 96 DNA Blood Handbook*.

17. **Press “Start” to start sample processing.**
18. **After the samples have been processed, remove the plates and blocks as instructed by the display. Press “Start” after removing each plate or block.**

    The first item to be removed contains the purified samples.

    Note: For short-term storage of up to 24 hours, we recommend storing the purified viral DNA and RNA at 2–8ºC. For long-term storage of over 24 hours, we recommend storage at –20ºC.

19. **Press “Stop” after all plates and blocks are removed.**
20. **Discard used plates, blocks, and 96-rod cover according to your local safety regulations.**

    Note: See “Safety Information” in the *BioSprint 96 DNA Blood Handbook*.

21. **Switch off the BioSprint 96 at the power switch.**
22. **Wipe the worktable and adjacent surfaces using a soft cloth or tissue moistened with distilled water or detergent solution. If infectious material is spilt on the worktable, clean using 70% ethanol or other disinfectant.**

    Note: Do not use bleach as disinfectant. See “Safety Information” in the *BioSprint DNA 96 Blood Handbook.*

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QIAGEN kit handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor.
Selected kit handbooks can be downloaded from www.qiagen.com/literature/handbooks/default.aspx .
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp .