User-developed protocol

User-Developed Protocol:

For simultaneous purification of viral DNA and RNA from veterinary samples such as serum, plasma, and other cell-free body fluids using the BioSprint 15 workstation

This protocol has been adapted by customers and is for purification of viral nucleic acids from veterinary samples such as serum, plasma, and other cell-free body fluids using the MagAttract® Virus Mini M48 Kit in combination with the BioSprint 15 workstation. **The procedure has not been thoroughly tested and optimized by QIAGEN.**

Introduction

The BioSprint 15 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 MagAttract Virus Mini M48 protocol. The MagAttract Virus Mini M48 Kit can be used to purify nucleic acids from a broad range of DNA and RNA viruses. However, kit performance is not guaranteed for each virus species and has to be validated by the user. **This protocol must not be used for processing human sample material on the BioSprint 15. This protocol is not for use in diagnostic procedures.**

IMPORTANT: Please read the *MagAttract Virus Mini M48 Handbook*, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure. Ensure that you are familiar with operating the BioSprint 15. See the *BioSprint 15 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 15 workstation, cat. no. 9000850
- "BS15 Viral NA Vet" protocol, available from QIAGEN Technical Services or your local distributor
- BioSprint 15 Plasticware (130), cat. no. 1030058
- Microcentrifuge tubes (1.5 ml) for lysis
- Heating block for 1.5 ml microcentrifuge tubes
- MagAttract Virus Mini M48 Kit, cat. no. 955336

- Ethanol (96–100%)*
- Isopropanol
- Pipettors and sterile, RNase-free pipet tips with aerosol barriers (20–1000 µl)
- Tubes for storage of purified nucleic acids
- Soft cloth or tissue and 70% ethanol or other disinfectant to clean worktable
- Disposable gloves

Preparing reagents

Dissolving carrier RNA and adding to Buffer AL

Add 1350 µl Buffer AVE to the tube containing 1350 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly, divide it 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 AVE and then added to Buffer AL.

Add carrier RNA to Buffer AL. The purification procedure is optimized so that 2 µg carrier RNA is added per sample. For 15 samples, add 40 µl carrier RNA to 3.96 ml Buffer AL. After adding RNA–Buffer AVE mix to Buffer AL, gently mix by inverting the tube 10 times. To avoid foaming, do not vortex.

Reconstituting QIAGEN Protease

Despite the instructions on the bottle, add **2.75 ml Buffer AVE** to a vial of lyophilized QIAGEN Protease to give a concentration of **2x Protease solution**. Label the vial accordingly. Protease Resuspension Buffer should not be used in this protocol. 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.

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.

* Do not use denatured alcohol, which contains other substances such as methanol or methyethylketone.

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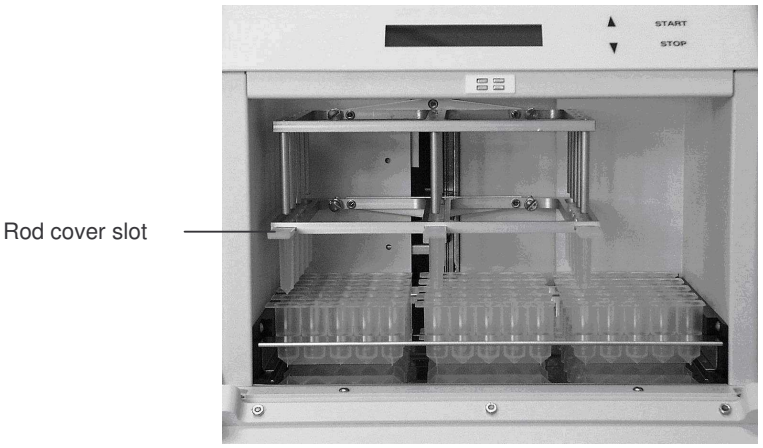
Things to do before starting

- Ensure that MagAttract Suspension B is fully resuspended. Vortex for at least 3 minutes before the first use, and for 1 minute before subsequent uses.
- Ensure that QIAGEN Protease, Buffer AW1, and Buffer AW2 have been prepared according to the instructions on page 2.
- Add carrier RNA reconstituted in Buffer AVE to Buffer AL according to the instructions on page 2.
- Load up to three 5-rod covers into the rod cover slots. There must always be a 5-rod cover above a column of 5-tube strips.

  Note: If necessary, remove the tube strip tray to allow easier loading of the 5-rod covers.

Rod Cover Slot



Insert a 5-rod cover into a rod cover slot so that the short tab faces inward and the long tab faces outward. 5-rod covers must be inserted so that they click into place.

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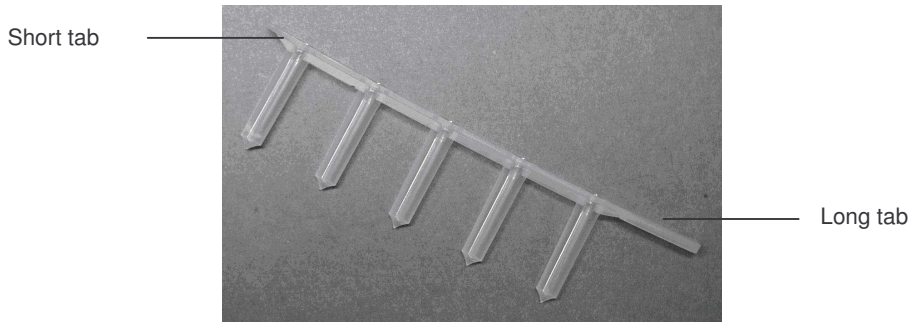
Tabs of the 5-Rod Cover



IMPORTANT: Do not push 5-rod covers further after they click into place, otherwise an instrument crash will occur.

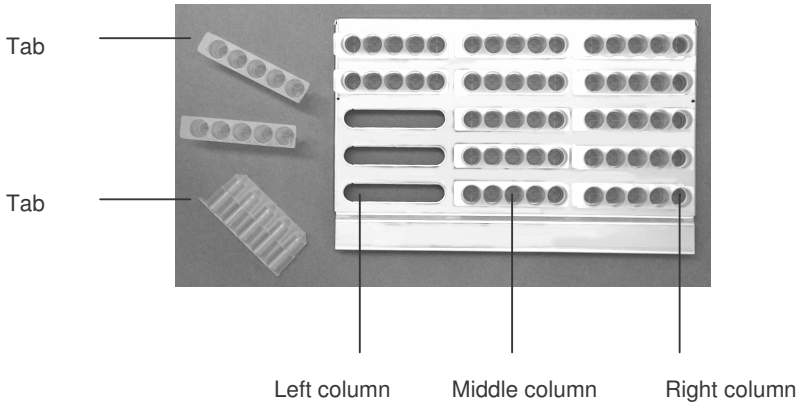
Procedure

1. **Switch on the BioSprint 15 at the power switch.**
2. **Open the front door of the BioSprint 15 and slide out the tube strip tray.**
3. **Load up to fifteen 5-tube strips into the tube strip tray. One 5-tube strip is used per sample.**

   If loading five 5-tube strips or fewer, we recommend loading them as a single column. If loading ten 5-tube strips or fewer, we recommend loading them as 2 columns.

   Load the 5-tube strips in the tube strip tray so that the tab of each 5-tube strip faces to the left. Make sure that the 5-tube strips are fully inserted into the tray and are not skewed.

Correct Loading of 5-Tube Strips in the Tube Strip Tray



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4. **Add reagents into each 5-tube strip according to the table below.**

Well	Reagent	Volume to add per well (µl)
1	Lysate*	695
2	Buffer AW1	700
3	Buffer AW2	500
4	Ethanol (96–100%)	500
5	Buffer AVE	75

** Added at step 10; volume of lysate includes QIAGEN Protease, Buffer AL, MagAttract Suspension B, and isopropanol.*

Note: Well 1 is at the left of the 5-tube strip, well 5 is at the right.

5. **Pipet 25 µl QIAGEN Protease into the bottom of a 1.5 ml microcentrifuge tube (not supplied). Add 200 µl sample to the QIAGEN Protease.**
6. **Add 200 µl Buffer AL containing carrier RNA, and mix by pulse-vortexing for 15 s.**
7. **Incubate at 56ºC for 15 min.**
8. **Add 20 µl MagAttract Suspension B and 250 µl isopropanol, and mix by pulse-vortexing for 15 s.**

   Note: Before adding MagAttract Suspension B, ensure that it is fully resuspended. Vortex for 3 min before using for the first time, and for 1 min before subsequent uses.
9. **Incubate at 56ºC for 5 min.**
10. **Transfer 695 µl of the lysate into well 1 of the 5-tube strip.**

    Note: Well 1 is at the left of the 5-tube strip, well 5 is at the right.

    Note: If processing more than one sample, record in which 5-tube strips you load the samples.
11. **Slide the tube strip tray fully into the BioSprint 15.**
12. **Close the front door of the BioSprint 15.**

    Closing the front and top doors protects the samples from contamination.
13. **Select the protocol "BS15 Viral NA Vet" using the ▲ and ▼ keys on the BioSprint 15 workstation. Press "START" to start the protocol run.**

    Warning: Avoid contact with moving parts during operation of the BioSprint 15. See the *BioSprint 15 User Manual* for safety information.
14. **After the protocol run ends, press "STOP" and slide out the tube strip tray. Transfer the eluted nucleic acids from well 5 of each 5-tube strip to other tubes for long-term storage.**

    Note: Well 5 is at the right of the 5-tube strip.

15. **Remove the 5-tube strips and 5-rod covers and discard them according to your local safety regulations.**

    Note: See “Safety Information” in the *MagAttract Virus Mini M48 Handbook*.

16. **Switch off the BioSprint 15 at the power switch.**
17. **Wipe the surface of the tube strip tray and adjacent surfaces with a soft cloth or tissue moistened with distilled water or a mild detergent solution. If infectious agents are spilt onto the tube strip tray, clean using 70% ethanol or other disinfectant.**

    Note: Do not use bleach as disinfectant. See “Safety Information” in the *MagAttract Virus Mini M48 Handbook*.