User-Developed Protocol

For manual purification of SBV RNA from bull semen

This protocol has been adapted by customers and is intended as a guideline for the purification of SBV RNA from bull semen using QIAamp® Viral RNA Mini Kit and QIAzol® Lysis Reagent. **This protocol has not been thoroughly tested and optimized by QIAGEN.**

IMPORTANT: Please read the "Safety Information" and "Important Notes" sections in the *QIAamp Viral RNA Mini Kit Handbook* and the *QIAzol Lysis Reagent Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (SDSs), available from the product supplier. QIAamp Viral RNA Mini Kit and QIAzol Lysis Reagent are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

Equipment and reagents to be supplied by user for all protocols

- QIAamp Viral RNA Mini Kit (cat. no. 52904 or 52906)
- QIAzol Lysis Reagent (cat. no. 79306)
- Pipets and disposable pipet tips with aerosol barriers (20–1000 µl)
- Microcentrifuge tubes (2 ml) for lysis and elution
- Microcentrifuge
- Chloroform
- Ethanol (96–100%)
- Vortexer

Procedure: Sample lysis

1. **Pipet 200 µl of sample into a 2 ml microcentrifuge tube.**
2. **Add 800 µl of QIAzol Lysis Reagent.**
3. **Homogenize the sample by pipetting the suspension up and down several times. Secure the cap of the tube containing the homogenate, and shake vigorously for 15 s.**
4. **Briefly centrifuge the 2 ml tube to remove droplets from the inside of the lid.**
5. **Place the tube containing the homogenate on the benchtop at room temperature (15–25°C) for 5 min.**
6. **Add 200 µl of chloroform.**
7. **Secure the cap of the tube containing the homogenate, and shake vigorously for 15 s.**
8. **Place the tube containing the homogenate on the benchtop at room temperature for 10 min.**
9. **Centrifuge at 12,000 x *g* for 10 min at 4°C.**
10. **Transfer 420 µl of the upper aqueous phase into a 2 ml microcentrifuge tube.**
    Avoid transferring organic solvents, they inhibit PCR.

At this point reagents of the QIAamp Viral RNA Mini Kit are to be utilized:

11. **Add 420 µl of ethanol (96–100%) to the sample, and mix by pulse-vortexing for 15 s. After mixing, briefly centrifuge the tube to remove droplets from the inside of the lid.**
12. **Carefully apply 630 µl of solution from step 11 to the QIAamp Mini column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini column into a clean 2 ml collection tube, and discard the tube containing the filtrate.**

    If the solution has not completely passed through the membrane, centrifuge again at a higher speed until all of the solution has passed through.
13. **Carefully open the QIAamp Mini column, and repeat step 12.**
14. **Carefully open the QIAamp Mini column, and add 500 µl of Buffer AW1. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.**

    It is not necessary to increase the volume of Buffer AW1 even if the original sample volume was larger than 140 µl.
15. **Carefully open the QIAamp Mini column, and add 500 µl of Buffer AW2. Close the cap and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min. Continue directly with step 17, or to eliminate any chance of possible Buffer AW2 carryover, perform step 16, and then continue with step 17.**

    Note: Residual Buffer AW2 in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, resulting in flow-through, containing Buffer AW2, contacting the QIAamp Mini column. Removing the QIAamp Mini column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp Mini column. In these cases, the optional step 16 should be performed.
16. **Recommended: Place the QIAamp Mini column in a new 2 ml collection tube (not provided), and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.**
17. **Place the QIAamp Mini column in a clean 1.5 ml microcentrifuge tube (not provided). Discard the old collection tube containing the filtrate. Carefully open the QIAamp Mini column and add 60 µl of Buffer AVE equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min.**
18. **Centrifuge at 6000 x *g* (8000 rpm) for 1 min.**

    A single elution with 60 µl of Buffer AVE is sufficient to elute at least 90% of the viral RNA from the QIAamp Mini column. Performing a double elution using 2 x 40 µl of Buffer AVE will increase yield by up to 10%. Elution with volumes of less than 30 µl will lead to reduced yields and will not increase the final concentration of RNA in the eluate.

    Viral RNA is stable for up to one year when stored at –20°C or –70°C.