EU FP VII PROJECT "VITAL"

STANDARD OPERATING PROCEDURE

SOP VITAL 010

Nucleic acids extraction from faeces, animal derived fertilizer, or blood

CREATED:	REVISED:	APPROVED:
David Rodriguez-Lazaro: 19/04/2010	FERA: 20/04/2010	Wim Van der Poel: 20/04/2010

— WARNING —

Adenoviruses, noroviruses, and hepatitis A and E viruses are viral pathogens. All samples and controls shall be handled by trained staff in a laboratory with appropriate equipment. Staff must be fully vaccinated against Hepatitis A and poliovirus. Persons using this SOP must be familiar with normal virology laboratory practice. This SOP does not presume to address fully all of the safety issues associated with its use. It is the responsibility of the user to establish appropriate health and safety practices and to ensure compliance with any national regulatory conditions.

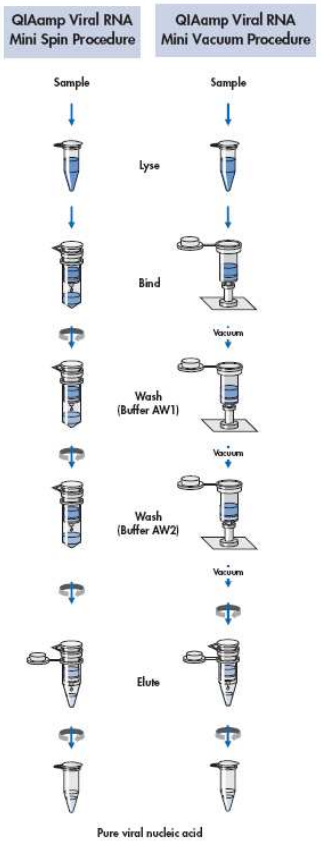
AIM

To extract the nucleic acids from extracts of faeces, animal derived fertilizer, or blood (see SOPs VITAL 001, 003, and 008 which refer to how to obtain the extracts).

PRINCIPLE

This protocol is based on the QIAamp® viral RNA mini kit (QIAGEN)[1].

The protocol is based on the selective binding properties of a silica gel-based membrane. The sample is first lysed under highly denaturing conditions to inactivate RNases and to ensure isolation of intact viral RNA. Buffering conditions are then adjusted to provide optimum binding of the RNA to the QIAamp® membrane, and the sample is loaded onto the QIAamp® Mini spin column. The RNA binds to the membrane, and contaminants are efficiently washed away in two steps using two different wash buffers. High-quality RNA is eluted in a special RNase-free buffer, ready for direct use or safe storage. The purified RNA is free of protein, nucleases, and other contaminants and inhibitors.



[1] QIAGEN (2007) QIAamp® Viral RNA Mini Handbook.

EQUIPMENT

- Micropipettes of a range of sizes, 1000µl, 200µl and 20µl
- Sterile, RNase-free micropipette tips of a range of sizes, 1000µl, 200µl and 20µl
- Gloves
- 1.5-ml microcentrifuge tubes.
- Microcentrifuge
- Refrigerator
- Freezer

REAGENTS

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

- QIAamp® viral RNA mini kit (QIAGEN). See page 42 of the QIAamp® Viral RNA Mini Handbook for ordering information
- Ethanol (96–100%). Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

PROCEDURE

1. At first use of the kit, prepare the carrier RNA solution, buffer AW1 and buffer AW2 according to the QIAamp® Viral RNA Mini kit handbook
2. Pipet 560 µl of Buffer AVL containing carrier RNA (1:100) into a 1.5 ml microcentrifuge tube.
   NOTE: If the sample volume is larger than 140 µl, increase the amount of Buffer AVL–carrier RNA proportionally (e.g., a 280 µl sample will require 1120 µl Buffer AVL–carrier RNA) and use a larger tube.
3. Add 140 µl of the faeces extract (see SOP VITAL 001), animal derived fertilizer extract (see SOP VITAL 003), or separated serum (see SOP VITAL 008) into the microcentrifuge tube. Mix by pulse-vortexing for 15 s.
   NOTE: To ensure efficient lysis, it is essential that the sample is mixed thoroughly with Buffer AVL to yield a homogeneous solution. Frozen samples that have only been thawed once can also be used.
4. Incubate at room temperature (15–25°C) for 10 min.
5. Briefly centrifuge the tube to remove drops from the inside of the lid.
6. Add 560 µl of ethanol (96–100%) to the sample, and mix by pulse-vortexing for 15 s. After mixing, briefly centrifuge the tube to remove drops from inside the lid.
   NOTE: If the sample volume is greater than 140 µl, increase the amount of ethanol proportionally (e.g., a 280 µl sample will require 1120 µl of ethanol). In order to ensure efficient binding, it is essential that the sample is mixed thoroughly with the ethanol to yield a homogeneous solution.
7. Carefully apply 630 µl of the solution from step 6 to the QIAamp® Mini column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6,000 × *g* (8,000 rpm) for 1 min. Place the QIAamp® Mini column into a clean 2 ml collection tube, and discard the tube containing the filtrate.

   NOTE: Buffer AVL contains guanidinium. Therefore dispose the filtrate in a high pH environment (e.g. into a 500 ml waste flask that contains 10 g of NaOH)

8. Carefully open the QIAamp® Mini column, and repeat step 7.
   NOTE: If the sample volume was greater than 140 µl, repeat this step until all of the lysate has been loaded onto the spin column.

9. Carefully open the QIAamp® Mini column, and add 500 µl of Buffer AW1. Close the cap, and centrifuge at 6,000 × *g* (8,000 rpm) for 1 min. Place the QIAamp® Mini column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.
   NOTE1: It is not necessary to increase the volume of Buffer AW1 even if the original sample volume was larger than 140 µl.
   NOTE2: Buffer AW1 contains guanidinium. Therefore dispose the filtrate in a high pH environment

10. Carefully open the QIAamp® Mini column, and add 500 µl of Buffer AW2. Close the cap and centrifuge at full speed (20,000 × g; 14,000 rpm) for 3 min.

    NOTE: Buffer AW2 does not contain guanidinium

11. Place the QIAamp® Mini column in a new 2 ml collection tube, and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.

Pig chain

12A. Place the QIAamp® Mini column in a clean 1.5 ml microcentrifuge tube. Discard the old collection tube containing the filtrate. Carefully open the QIAamp® Mini column and add 50 µl of Buffer AVE equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min. Centrifuge at 6,000 × *g* (8,000 rpm) for 1 min.

13A. Carefully open the QIAamp® Mini column and add other 50 µl of Buffer AVE equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min. Centrifuge at 6,000 × *g* (8,000 rpm) for 1 min. The final nucleic acid eluate will be 100 µl

Vegetables chain

12B. Place the QIAamp® Mini column in a clean 1.5 ml microcentrifuge tube. Discard the old collection tube containing the filtrate. Carefully open the QIAamp® Mini column and add 150 µl of Buffer AVE equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min. Centrifuge at 6,000 × *g* (8,000 rpm) for 1 min.

13B Carefully open the QIAamp® Mini column and add other 150 µl of Buffer AVE equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min. Centrifuge at 6,000 × *g* (8,000 rpm) for 1 min. The final nucleic acid eluate will be 300 µl

14. Store the RNA solution at –20°C or –70°C.