April 2020 | EN

Supplementary Protocol

Manual Purification of Pathogen Nucleic Acids from Fluid Samples using the IndiSpin® QIAcube® HT Pathogen Kit

For the purification of viral RNA and DNA, DNA of easy-to-lyse bacteria from fluid samples, or pretreated tissue samples

Further information and support

- IndiSpin QIAcube HT Pathogen Kit Handbooks: **www.indical.com/handbooks**
- Technical assistance: **support@indical.com**

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Important points before starting

- All centrifugation steps are carried out at room temperature (18-25°C)

  Note: Do not use a precooled centrifuge.
- Dissolve Carrier RNA in Buffer AVE as indicated on the respective tube.
- Add Isopropanol (100%) to Buffer ACB and Ethanol (96-100%) to Buffers AW1 and AW2 before use. See the respective bottle labels for volumes.

  Equilibrate buffers to room temperature (15–25ºC).

INDICAL BIOSCIENCE

Equipment and reagents

- IndiSpin QIAcube HT Pathogen Kit (SP54161, INDICAL)
- 2ml 96-Well-Deep-Well Plates (cat. no. PW940187 INDICAL; cat. no. 030501306; Eppendorf)

  Optional (when using PW940187): Pipetting Aid[1]
- Universal laboratory centrifuge with Plate Rotor 2 x 96, for 96-Well-Deep-Well Plates and QIAamp 96 plate on top (e.g. Item No: B34183 and 368690; Beckman Coulter)
- **Optional**: AirPore Tape Sheet (e.g. cat. no. 19571, QIAGEN)

Procedure

1. Pipet 20 µl Proteinase K into the bottom of a 96-Well-Deep-Well Plate well and add 200 µl sample.
   Note: If your sample volume is less than 200 µl bring it to 200 µl by adding PBS.

2. Add 180 µl Buffer VXL to each sample in the 96-Well-Deep-Well Plate.
   Note: For cell-free samples, ensure that 1 µg Carrier RNA is added per 180 µl Buffer VXL.

3. Incubate at 20-25°C for 15 min on a microplate shaker.

4. Add 350 µl Buffer ACB to each sample in the 96-Well-Deep-Well Plate.

5. Pipet up and down several times to mix lysate.

6. Place two QIAamp 96 plates on top of 96-Well-Deep-Well Plates. Mark the QIAamp 96 plates for later sample identification.

7. Carefully transfer the lysis mixture (maximum 750 µl) of each sample from step 4 to each well of the QIAamp 96 plates.

8. Centrifuge for 4 min at 6000 x *g*.

   Optional: Seal each QIAamp 96 plate with an AirPore Tape Sheet.

   Note: After centrifugation, check that all of the lysate has passed through the membrane in each well of the QIAamp 96 plates. If lysate remains in any of the wells, centrifuge for a further 4 min.

[1] Contact your local Sales Representative or support@indical.com for further information

9. Place each QIAamp 96 plate in the correct orientation on a new 96-Well-Deep-Well Plate. Discard used 96-Well-Deep-Well Plates.

10. Carefully add 600 µl Buffer AW1 to each sample.

11. Centrifuge for 2 min at 6000 x *g*.

    Optional: Seal each QIAamp 96 plate with an AirPore Tape Sheet.

    Note: After centrifugation, check that all of the buffer has passed through the membrane in each well of the QIAamp 96 plates. If lysate remains in any of the wells, centrifuge for a further 2 min

12. Carefully add 600 µl Buffer AW2 to each sample.

13. Centrifuge for 15 min at 6000 x *g*.

    Note: **Do not seal the plate with AirPore Tape**. The heat generated during centrifugation ensures evaporation of residual ethanol in the sample (from Buffer AW2) that might otherwise inhibit downstream reactions.

14. Place each QIAamp 96 plate in the correct orientation on a new 96-Well-Deep-Well Plate.

15. Add 100 µl Buffer AVE to each sample, incubate for 1 min at room temperature (15–25°C). Centrifuge for 4 min at 6000 x *g*.

Product Sheet	Version	Change
HB-2548-EN-001	April 2020	INDICAL Design