IndiSpin Pathogen Kit
Quick Start Protocol

For manual use.

The IndiSpin Pathogen Kits (cat. no. SP54104 and SP54106) can be stored at room temperature (15-25°C). For expiry date information, read the label on the kit box.

Further information and support

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

Important notes before starting

- Dissolve carrier RNA in Buffer AVE as indicated on the 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.
- Carry out all centrifugation steps at room temperature in a conventional table-top microcentrifuge.
- Equilibrate buffers to room temperature (15-25ºC).

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Procedure

1. Pipet 20 µl Proteinase K into a 2 ml microcentrifuge tube (not provided).
2. Add 200 µl fluid sample to the Proteinase K.

   Note: If your sample volume is less than 200 µl, bring it to 200 µl by adding PBS.
3. Add 100 µl Buffer VXL. Close the cap and mix by pulse vortexing.

   Note: For cell-free samples, ensure that 1 µg Carrier RNA is added per 100 µl Buffer VXL.
4. Incubate at 20-25°C for 15 min.
5. Briefly centrifuge the tube to remove drops from the inside of the lid.
6. Add 350 µl Buffer ACB to the sample and mix thoroughly by pulse-vortexing.
7. Briefly centrifuge the tube to remove drops from the inside of the lid.
8. Transfer the lysate to a spin column placed in a 2 ml collection tube. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Transfer the spin column to a clean 2 ml collection tube and discard the collection tube containing the filtrate.
9. Add 600 µl Buffer AW1 and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Transfer the spin column to a clean 2 ml collection tube and discard the collection tube containing the filtrate.
10. Add 600 µl Buffer AW2 and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Transfer the spin column to a clean 2 ml collection tube and discard the collection tube containing the filtrate.
11. Centrifuge the spin column at 20,000 x *g* (14,000 rpm) for 2 min.
12. Place the spin column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube containing the filtrate. Add 50-150 µl Buffer AVE to the center of the membrane, close the cap and incubate at room temperature for 1 min.
13. Centrifuge at 20,000 x *g* (14,000 rpm) for 1 min.