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IndiSpin® Pathogen Kit Handbook

For the purification of viral RNA and DNA and bacterial DNA from animal whole blood, serum, plasma, other body fluids, swabs and washes, and tissue

REF IndiSpin Pathogen Kit (cat no SP54104),
formerly QIAamp® cador® Pathogen Mini Kit (50)

REF IndiSpin Pathogen Kit (cat no SP54106),
formerly QIAamp cador Pathogen Mini Kit (250)

Manufactured by QIAGEN® GmbH for INDICAL BIOSCIENCE
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INDICAL BIOSCIENCE

Contents

Kit contents

IndiSpin Pathogen Kit	**(50)**	**(250)**
Cat. no.	**SP54104**	**SP54106**
Number of preps	**50**	**250**
IndiSpin Columns	50	250
Buffer VXL[1]	1 x 6 ml	1 x 30 ml
Buffer ACB (concentrate)[1,2]	1 x 12 ml	1 x 60 ml
Proteinase K	1 x 1.25 ml	1 x 6 ml
Carrier RNA (poly A)	1 x 310 µg	1 x 310 µg
Buffer AW1 (concentrate)[1,3]	1 x 19 ml	1 x 98 ml
Buffer AW2 (concentrate)[3]	1 x 17 ml	1 x 81 ml
Buffer AVE	1 x 20 ml	2 x 20 ml
Quick-Start Protocol (PCard)	1	1

1 CAUTION: Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 5 for safety information.
2 Before using for the first time, add isopropanol as indicated on the bottle to obtain a working solution.
3 Before using for the first time, add ethanol (96-100%) as indicated on the bottle to obtain a working solution.

Storage

IndiSpin columns and buffers can be stored dry at room temperature (15-25ºC) until the expiration date stated on the kit box without affecting performance.

Lyophilized Carrier RNA can be stored at room temperature until the expiration date stated on the kit box. For use, lyophilized Carrier RNA should be dissolved in Buffer AVE and then added to Buffer VXL, as described in “Preparing reagents”, on page 17. This Carrier RNA/Buffer AVE/Buffer VXL mix solution should be prepared fresh and is stable at room temperature for up to 48 hours. Unused Carrier RNA dissolved in Buffer AVE should be immediately frozen in aliquots at -30 to -15°C. Do not subject aliquots of Carrier RNA to more than 3 freeze-thaw cycles.

Proteinase K can be stored at room temperature (15-25°C). To store for extended periods of time, or if the ambient temperature often exceeds 25°C, we recommend storing at 2-8°C.

Intended use

The IndiSpin Pathogen Kit is intended for the extraction of pathogen nucleic acids (viral RNA and DNA, and bacterial DNA) from animal whole blood, serum, plasma, other body fluids, swabs, washes, and tissue (homogenate).

For molecular biology applications.

Symbols

 Legal manufacturer

 Lot number

 Use by date

 Temperature limitations for storage

 Handbook

 Catalog number

 Material number

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available from your local sales representative or by Email request under **compliance@indical.com**.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Buffer VXL and Buffer AW1 contain guanidine hydrochloride, and Buffer ACB contains guanidine thiocyanate, which can form highly reactive compounds if combined with bleach.

If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality control

In accordance with INDICAL's ISO-certified Quality Management System, each lot of IndiSpin Pathogen Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The IndiSpin Pathogen Kit enables the efficient purification of viral RNA and DNA, as well as bacterial DNA, from a broad range of animal sample types, including whole blood, serum, plasma, swabs, washes, and tissue (see “Starting material” on page 12). The extracted nucleic acids are free of proteins, nucleases, and other impurities, and are ready for use in downstream applications, such as real-time PCR-based pathogen identification.
However, specific combinations of sample types and pathogens should be validated by the user.

The kit is not intended for host RNA or host DNA preparation.

Principle and procedure

Samples are lysed under highly denaturing conditions at room temperature (15 - 25°C) in the presence of Proteinase K and Buffer VXL, which together ensure the inactivation of nucleases. Adding Buffer ACB adjusts the binding conditions for the copurification of DNA and RNA. The lysate is then transferred to an IndiSpin Column. During centrifugation, nucleic acids are adsorbed onto the silica membranes while contaminants pass through. Two efficient wash steps remove the remaining contaminants and enzyme inhibitors, and nucleic acids are eluted in Buffer AVE.

Performance is not guaranteed for every combination of starting material and pathogen species and must be validated by the user. Some samples may require a pretreatment (see Table 1, page 9).

Nucleic acid purification protocol

The “Purification of pathogen nucleic acids from fluid samples” protocol (page 21) is optimized for purification of viral RNA and DNA, and the DNA of easy-to-lyse bacteria from up to 200 µl of fluid material. Suitable starting materials for **direct processing** using this method include:

- whole blood
- serum
- plasma
- oral fluid
- body cavity fluids (e.g., peritoneal, synovial, cerebrospinal)
- liquid extracts from swabs (e.g., nasal, pharyngeal, and cloacal* swabs)
- wash fluids (e.g., from bronchoalveolar lavages)
- other fluids, such as urine or feces suspensions*

Most sample types can be directly processed without pretreatment. However, depending on the starting material and the target pathogen, one of the pretreatment protocols may be needed. For samples that require a pretreatment prior to nucleic acid purification, Table 1 on page 9 provides an overview of which pretreatment protocols are suited to different starting material and pathogen combinations.

The lysis and binding solutions used in the procedure are Buffer VXL and Buffer ACB. Please pay attention to the information given under “Safety information”, page 5.

* The processing of samples with a high inhibitor content, such as urine and feces, may require a reduction in sample input volume or further measurements. For further pretreatment recommendations, contact INDICAL support (**support@indical.com**).

Pretreatments

The pretreatments mentioned in this handbook are optimized for specific combinations of starting material and target pathogens. The choice of pretreatment depends on the workflow focus, and is to be followed by nucleic acid purification.

Table 1 on page 9 summarizes the pretreatments and their applications.

Some of the pretreatments may require additional components, which are indicated in each pretreatment protocol.

Table 1: Overview of available pretreatment protocols

Sample	Target	Pretreatment	handbook
Fluids (e.g., whole blood, serum, plasma, swab or wash fluid, pretreated tissue)	Viral RNA and DNA, DNA of easy-to-lyse bacteria[1]	-	-
Whole blood or pretreated tissue	DNA of difficult-to-lyse bacteria[1]	**Pretreatment B1** for difficult-to-lyse bacteria in whole blood or pretreated tissue	**HB-2533**
Serum, plasma, swabs, washes, body cavity fluids, urine	DNA of difficult-to-lyse bacteria[1]	**Pretreatment B2** for difficult-to-lyse bacteria in body fluids[2]	**HB-2534**
High volume cell-free fluids (for increased sensitivity)	DNA of easy-to-lyse bacteria[1]	**Pretreatment B3** for difficult-to-lyse bacteria in body fluids[2]	**HB-2549**

Tissue (e.g., liver, spleen, kidney, lymph node)	Pathogen nucleic acids	**Pretreatment T1** Mechanical disruption of tissue	**HB-2535**
	Viral DNA[3], bacterial DNA[4]	**Pretreatment T2** Enzymatic digestion of tissue	**HB-2536**
Rapid Partial Disruption of tissue	Viral RNA and DNA, DNA of easy-to-lyse bacteria[1]	**Pretreatment T3**	**HB-2537**
Tissue containing high amount of lipids and/or nucleases (e.g. brain, pancreas)	Viral RNA and DNA, DNA of easy-to-lyse bacteria[1]	**Pretreatment T4**	**HB-2538**
Feces	Viral RNA and DNA	**Pretreatment F1** Non-lysing suspension method	**HB-2513**
	Bacterial DNA[1] and viral DNA	**Pretreatment F2** Lysing suspension method	**HB-2514**
	Mycobacterium avium subsp. *paratuberculosis* (MAP) DNA	**Pretreatment F-MAP**	**HB-2503**
Filter paper cards		**Pretreatment C1**	**HB-2520**
Swabs (tracheal, oropharyngeal, blood)		**Pretreatment S1**	**HB-2516**

1 Gram-positive bacteria are difficult to lyse due to their rigid cell wall. Many Gram-negative bacteria are easy to lyse, but some are not and will also benefit from Pretreatment B1 or B2.
2 Not suitable for whole blood.
3 Not suitable for viral RNA as the lysis conditions do not sufficiently conserve RNA integrity.
4 For difficult-to-lyse bacteria, subsequently use Pretreatment B1.

For further information on Pretreatments website www.indical.com/handbooks contact INDICAL Support at **support@indical.com**.

Equipment and reagents to be supplied by user

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.

- Pipettors and disposable pipette tips with aerosol barriers (20–1000 µl)
- Multichannel pipettor and disposable 1000 µl pipette tips with aerosol barriers
- Multidispenser
- Ethanol (96-100%)*
- Isopropanol
- Phosphate-buffered saline (PBS), may be required for diluting samples
- Vortexer

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

Important notes

Starting material

Do not overload the IndiSpin Column, as this can lead to impaired nucleic acid extraction and/or performance in downstream assays. For samples with very high host nucleic acid contents (e.g., for certain tissues, such as spleen or blood samples with highly increased cell counts), use less than the maximum amount of sample recommended in the protocol or pretreatments. In some downstream applications such as PCR and RT-PCR, very high background concentrations of nucleic acids may impair the reaction. Use appropriate controls (e.g., an internal control) to verify successful PCR amplification.

Avoid transferring material to the IndiSpin Column that could subsequently clog the membrane (e.g., blood clots, solid tissue, swab fibers).

Highly viscous fluids may require treatment to reduce their viscosity, to allow for efficient extraction of pathogen nucleic acids. Please contact INDICAL support at **support@indical.com** for recommendations.

Avoid repeated thawing and freezing of samples, since this may reduce nucleic acid yield and quality.

Animal whole blood

Blood samples treated with EDTA, citrate, or heparin as anticoagulant can be used for nucleic acid purification. Samples can be either fresh or frozen, provided that they have not been freeze-thawed more than once. Freeze-thawing more than once can lead to denaturation and precipitation of proteins, resulting in potential reduction in viral titers, and therefore, reduced yields of viral nucleic acids.

After collection and centrifugation, whole blood samples can be stored at 2-8ºC for up to 6 hours. For longer storage, we recommend freezing aliquots at -30 to -15ºC or at -70ºC.

We recommend using 50-200 µl blood containing non-nucleated erythrocytes. However, highly elevated cell counts due to inflammatory or neoplastic diseases may strongly increase the host nucleic acid content of a sample. In this case, reduction of sample input to 50 µl may improve results in downstream assays, particularly in RT-PCR. If using less than 200 µl blood, adjust the sample volume to 200 µl with PBS or 0.9% NaCl.

For blood samples containing nucleated erythrocytes (e.g., samples from bird and fish), use less than 50 µl blood and adjust the sample volume to 200 µl with PBS or 0.9% NaCl.

Animal serum, plasma, other body fluids, swab, and wash specimens

Frozen plasma or serum must not be thawed more than once before processing.

We recommend storing swabs in transport media; for example, viral transport media (VTM) or brain-heart infusion broth (BHI). Remove the swab and squeeze out the liquid by pressing the swab against the inside of the storage tube. For extraction of viral RNA or DNA, we recommend centrifuging the swab media briefly to ensure any residual solid materials are removed.

Note: Solid pieces remaining in the sample fluid may aggregate on the IndiSpin Column, which may decrease nucleic acid yield.

Up to 200 µl serum, plasma, other body fluid, swab media supernatant, or wash fluid can be processed.

Carrier RNA must be used in the nucleic acid purification protocol to prevent the loss of nucleic acids during the procedure (see page 15 for information on the use of Carrier RNA).

The processing of samples with very high inhibitor contents, such as urine or fecal suspensions, may require a reduction in sample input volume and/ or an extra pretreatment to remove inhibitors. To reduce the input volume, use 25-50 µl of the sample and adjust the volume to 200 µl with PBS or 0.9% NaCl.

For extraction of bacterial DNA, the input volume can be increased to more than 200 µl, e.g., 1.5 ml for increased sensitivity of bacterial detection. Gram-negative bacteria in cell-free fluids can be concentrated by centrifugation of higher volumes. Resuspend pellets in PBS and use 200 µl as starting volume. See Pretreatment B2 for extraction of DNA from difficult-to-lyse bacteria and Pretreatment B3 for extraction of DNA from easy-to-lyse bacteria.

Animal tissues

When working with tissue samples, mechanical or enzymatic disruption of the tissue structure is the prerequisite for liberation of cells, subsequent release of nucleic acids, and membrane permeability of the material.

Different tissue types can vary widely with regard to texture and rigidity, cell types, and content of host nucleic acids and inhibitory substances. In addition, the localization of pathogen nucleic acids in the tissue may vary depending on tissue type, pathogen, and stage of infection. Therefore, suitability of the pretreatment protocols in this handbook should be evaluated for each new combination of tissue and pathogen. Additional pretreatments for tissue samples are available at INDICAL Support, including a rapid protocol and recommendations for difficult tissues.

Up to 25 mg of fresh or frozen tissue can be used as a starting amount. For tissues with a very high number of cells for a given mass of tissue, such as spleen, a reduced amount of starting material (5-10 mg) should be used.

Yields of nucleic acids

For samples containing a low amount of cells (e.g., serum and plasma), the yield of viral nucleic acids obtained can be below 1 µg and is therefore difficult to quantify using a spectrophotometer. In addition, eluates prepared with Carrier RNA may contain much more Carrier RNA than target nucleic acids. The IndiSpin Pathogen Kit recovers total nucleic acids. Therefore, cellular DNA and RNA will be co-purified from any cells in the sample along with viral RNA and DNA, and bacterial DNA, and cannot be distinguished using spectrophotometric measurements. We recommend using quantitative amplification methods such as quantitative real-time PCR or real-time RT-PCR to determine pathogen nucleic acid yields.

Using Carrier RNA and internal controls

Carrier RNA

We recommend adding Carrier RNA to fluids containing low amounts of cells such as serum, plasma, swab media, and wash fluid.
This enhances adsorption of viral RNA and DNA to the silica membranes, which is especially important when the target molecules are not abundant. In addition, an excess of Carrier RNA reduces the chances of viral RNA degradation in the rare event that RNases are not denatured by the chaotropic salts and detergents in the lysis buffer. Not using Carrier RNA may decrease the recovery of viral nucleic acids. Do not add Carrier RNA to whole blood and tissue samples, or other

samples containing a high amount of cells.

Internal Control

Use of an internal control, such as the intype IC-DNA or intype IC-RNA is optional, depending on the amplification system of choice. If the IndiSpin Pathogen Kit is used in combination with amplification systems that employ an internal control, introduction of these internal controls may be required during the purification procedure, to monitor the efficiency of sample preparation and downstream assay.

Add unprotected internal control nucleic acids (e.g., plasmid DNA or in vitro transcribed RNA) to VXL mixture only. Do not add these internal control nucleic acids directly to the sample.

The amount of internal control added depends on the assay system and the elution volume. Evaluation of the correct amount of internal control nucleic acid must be performed by the user. Refer to the manufacturer's instructions to determine the optimal concentration of internal control or contact INDICAL Support (**support@indical.com**) for further information.

Storing nucleic acids

For short-term storage of up to 24 hours, we recommend storing the purified viral RNA and DNA at 2-8°C. For storage longer than 24 hours, we recommend storing purified nucleic acids at -30 to -15°C, or even at -70°C in the case of RNA.

Handling RNA

RNases are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate

and only minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure.

Preparing reagents

Carrier RNA stock solution

For use, lyophilized Carrier RNA should first be dissolved in Buffer AVE. Add 310 µl Buffer AVE to the tube containing 310 µg lyophilized Carrier RNA to obtain a stock solution of 1 µg/µl. Add this solution to Buffer VXL mixture as in Table 5 on page 22. Unused Carrier RNA dissolved in Buffer AVE should be frozen in aliquots at -30 to -15°C. Aliquots of Carrier RNA should not be subjected to more than 3 freeze-thaw cycles.

Adding Carrier RNA to Buffer VXL

We recommend adding Carrier RNA to fluids containing a low amount of cells such as serum, plasma, swabs media, and wash fluid. Do not add Carrier RNA to samples with a high cell content such as whole blood and tissue because high amounts of background nucleic acids may negatively influence downstream applications such as RT-PCR.

Carrier RNA dissolved in Buffer AVE is added to Buffer VXL so that 1 µg carrier RNA is present in each sample.

Note: 100 µl of Buffer VXL containing dissolved Carrier RNA is used per preparation.

Important note: Carrier RNA does not dissolve in Buffer VXL. It must first be dissolved in Buffer AVE.

The Buffer VXL solution containing dissolved Carrier RNA should be prepared fresh and is stable at room temperature (15-25ºC) for up to 48 hours.

Proteinase K

The IndiSpin Pathogen Kit contains ready-to-use Proteinase K supplied in a specially formulated storage buffer. The activity of the Proteinase K solution is 600 mAU/ml.

Proteinase K is stable for at least 1 year after delivery when stored at room temperature (15-25°C). To store for more than 1 year or if ambient temperature often exceeds 25°C, we recommend storing Proteinase K at 2-8°C.

Buffer ACB

Buffer ACB is supplied as a concentrate. Before using for the first time, add Isopropanol (100%) as indicated on the bottle and in Table 2, page 18. Tick the check box on the bottle label to indicate that Isopropanol has been added. Mix well after adding Isopropanol.

Table 2: Preparation of Buffer ACB

No of preps	ACB concentrate	Isopropanol	Final volume
50	12 ml	8 ml	20 ml
250	60 ml	40 ml	100 ml

Buffer AW1

Buffer AW1 is supplied as a concentrate. Before using for the first time, add Ethanol (96-100%) as indicated on the bottle and in Table 3, page 19. Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW1 can be stored at room temperature
(15-25°C) for up to 1 year. Mix well after adding Ethanol.

Table 3: Preparation of Buffer AW1

No of preps	ACB concentrate	Ethanol	Final volume
50	19 ml	25 ml	44 ml
250	98 ml	130 ml	228 ml

Buffer AW2

Buffer AW2 is supplied as a concentrate. Before using for the first time add Ethanol (96-100%) as indicated on the bottle and in Table 4, page 19. Tick the check box on the bottle label to indicate that ethanol has been added. Mix well after adding Ethanol.

Table 4: Preparation of Buffer AW2

No of preps	ACB concentrate	Ethanol	Final volume
50	17 ml	40 ml	57 ml
250	81 ml	190 ml	271 ml

Handling Buffer AVE

Buffer AVE is RNase-free upon delivery. It contains sodium azide, an antimicrobial agent that prevents growth of RNase-producing organisms. However, as this buffer does not contain any RNase-degrading chemicals, it will not actively inhibit RNases introduced by

inappropriate handling. When handling Buffer AVE, take extreme care to avoid contamination with RNases. Follow general precautions for working with RNA, such as frequent change of gloves and keeping tubes closed whenever possible.

Protocol: Purification of pathogen nucleic acids from fluid samples

This protocol is for the purification of viral RNA and DNA, and the DNA of easy-to-lyse bacteria from fluid samples or pretreated tissue samples. The sample volume can be up to 200 µl.

Important points before starting

- Before beginning the procedure, read “Important notes” (page 12).
- Check that Buffer ACB, Buffer AW1, Buffer AW2, and Carrier RNA have been prepared according to the instructions in “Preparing reagents” (page 17).
- Check that Buffer VXL or Buffer ACB does not contain a white precipitate. If necessary, incubate Buffer VXL or Buffer ACB for 30 minutes at 37°C with occasional shaking to dissolve precipitate.

Things to do before starting

- If necessary, thaw and equilibrate samples at room temperature (15-25°C).
- If the volume of the sample is less than 200 µl, add PBS or 0.9% NaCl to a final volume of 200 µl.
- If necessary, prepare a mixture of Buffer VXL and Carrier RNA according to Table 5 on page 22, for use in step 3 of the procedure.

  Important: Do not add Proteinase K directly to the Buffer VXL mixture! This can cause clogs or precipitates. Follow the procedure as described below (pipetting Proteinase K into the wells, followed by sample and then Buffer VXL/Carrier RNA mixture).

Note: Prepare a volume of the Buffer VXL/Carrier RNA/Internal Control mixture that is 10% greater than that required for the total number of sample purifications to be performed.

Table 5: Buffer VXL mixture preparation

Reagent	Number of samples *		
	1	12	30
Buffer VXL	100 µl	1.32 ml	3.3 ml
Carrier RNA (1 µg/µl)	1 µl	13 µl	33 µl

* The volume prepared is 110% of the required volume to compensate for pipetting error and possible evaporation. Excess buffer should be discarded.

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 or 0.9% NaCl.
3. **Add 100 µl Buffer VXL. Close the cap and mix by pulse-vortexing.**

   To ensure sufficient lysis, thoroughly mix the sample and Buffer VXL to yield a homogenous solution. If using sample fluid containing Buffer ATL, e.g., after enzymatic digestion of tissue, precipitates may form. Precipitates can be dissolved by brief incubation at 56°C. However, they have no influence on subsequent protocol steps.

   Note: If processing cell-free samples, ensure that 1 µg Carrier RNA is added per 100 µl of Buffer VXL before use. Do not add Carrier RNA if processing cell-rich samples, such as whole blood and tissue.
4. **Incubate at 20-25°C for 15 min.**
5. **Briefly centrifuge the 2 ml tube to remove drops from inside the lid.**
6. **Add 350 µl Buffer ACB to the sample, close the cap, and mix thoroughly by pulse-vortexing.**

   Ensure that Isopropanol was added to the Buffer ACB concentrate before first use.
7. **Briefly centrifuge the 2 ml tube to remove drops from inside the lid.**
8. **Transfer the lysate from step 7 to the IndiSpin Column placed 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 IndiSpin**

Column into a clean 2 ml collection tube, and discard the collection tube containing the filtrate.

If the lysate has not completely passed through the column after centrifugation, centrifuge again at a higher speed (up to 20,000 x *g*; 14,000 rpm) until the IndiSpin Column is empty.

9. **Open the IndiSpin Column, and add 600 µl Buffer AW1 without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the IndiSpin Column into a clean 2 ml collection tube, and discard the collection tube containing the filtrate.**

10. **Open the IndiSpin Column, and add 600 µl Buffer AW2 without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the IndiSpin Column into a clean 2 ml collection tube, and discard the collection tube containing the filtrate.**

11. **Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 2 min to dry the membrane.**

12. **Place the IndiSpin Column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Open the IndiSpin Column, and add 50-150 µl Buffer AVE to the center of the membrane. Close the cap, and incubate at room temperature (15-25°C) for 1 min. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min.**

    Important: Ensure that the elution buffer is equilibrated at room temperature. If elution is performed with a small volume (<75 µl), the elution buffer must be dispensed onto the center of the membrane for complete elution of bound RNA and DNA. Elution volume is flexible and can be adapted according to requirements of the downstream application.

    To reduce noise, the centrifugation speed for elution can be set to 6000 x *g*. If this is done, the recovered eluate volume will be

approximately 5 µl less than elution buffer volume applied onto the column.

Troubleshooting guide

This troubleshooting guide may be helpful in solving any problems that may arise.

For more information or help please contact INDICAL Support at **support@indical.com**.

		Comments and suggestions
Little or no pathogen DNA or RNA in the eluate		
1	Buffer ACB prepared incorrectly	Check that Buffer ACB concentrate was diluted with the correct volume of Isopropanol, as indicated on the bottle. Use 100% Isopropanol. Repeat the purification protocol with new samples.
2	Buffer AW1 or Buffer AW2 prepared incorrectly	Check that Buffer AW or Buffer AW2 concentrate was diluted with the correct volume of 96–100% ethanol, as indicated on the bottle. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. Repeat the purification protocol with new samples.
3	Insufficient sample lysis	Proteinase K was stored at elevated temperatures for too long. Repeat the purification procedure using new samples and fresh Proteinase K (see storage recommendations on page 4). For some DNA viruses and bacteria, heated lysis may improve lysis efficiency. In this case, mix the samples thoroughly after addition of Proteinase K and incubate for 15 min at 70°C.
4	IndiSpin Column not incubated with Buffer AVE before elution	After addition of Buffer AVE, the IndiSpin Column should be incubated at room temperature (15-25°C) for 1 min.
5	Carrier RNA not added to Buffer VXL	For samples containing a low number of cells, not using Carrier RNA may decrease the recovery of pathogen nucleic acids. For these samples, reconstitute Carrier RNA in Buffer AVE and add reconstituted Carrier RNA too Buffer VXL as

		described on page 22. Repeat the purification procedure with new samples.
6	Degraded Carrier RNA	Carrier RNA reconstituted in Buffer AVE was not stored at -30 to -15°C or underwent multiple freeze-thaw cycles. Alternatively, Buffer VXL/Carrier RNS mixture was stored for more than 48 h at 2-8°C. Prepare a new tube of Carrier RNA dissolved in Buffer AVE. Repeat the purification procedure with new samples.
7	Buffer VXL/Carrier RNA mixture mixed insufficiently	Mix well by pipetting with a large pipette.
8	RNase contamination in Buffer AVE	If tubes containing Buffer AVE are accessed repeatedly, be careful to not introduce RNases which can degrade viral RNA. In case of RNase contamination, replace the open vial of Buffer AVE with a new vial. Repeat the purification procedure with new samples.
9	Nucleic acids in samples already degraded prior to purification	Samples were freeze-thawed more than once or stored at room temperature (15-25ºC) for too long. Always use fresh samples or samples thawed only once. Repeat the purification protocol with new samples.
DNA or RNA does not perform well in downstream applications		
1	Little or no DNA or RNA in the eluate	See “Little or no pathogen DNA or RNA in the eluate” (above) for possible reasons.
2	Excessive eluate in the amplification reaction	Some sample types may contain high amounts of background nucleic acids (e.g., animal whole blood, tissue) or PCR inhibiting substances (feces). High amounts of background nucleic acids may inhibit amplification reactions, and removal of inhibitors might not be complete without special treatment. Reduce the amount of sample input or/and the amount of eluate added to the amplification reaction.
3	Excessive Carrier RNA in the eluate	Determine the maximum amount of Carrier RNA suitable for your amplification reaction. Adjust the

		concentration of Carrier RNA solution added to the Buffer VXL accordingly.
4	Performance of purified nucleic acids in assays varies with aging of reconstituted wash buffers	Salt and ethanol components of Buffer AW1 or Buffer AW2 may have separated out after being left for a ling period between preparation. Always mix buffers thoroughly before each preparation.
5	Residual ethanol in the eluate	Use the drying step (step 11) in the protocol: “Purification of pathogen nucleic acids from fluid samples” (page 24).
Precipitate in buffers		
1	Precipitate in Buffer VXL or Buffer ACB	Precipitate may form after storage at low temperature or prolonged storage. To dissolve precipitate, incubate Buffer VXL or ACB for 30 min at 37°C, with occasional shaking.
2	Precipitate in sample-Buffer VXL mixture	If using sample fluid containing Buffer ATL, e.g., after enzymatic digestion of tissue, precipitate may form after addition of Buffer VXL to the sample. The precipitate does not influence subsequent protocol steps and can be dissolved by brief incubation at 56°C.

Order information

Product name	Cat. no.
IndiSpin Pathogen Kit (50) *formerly QIAamp cador Pathogen Mini Kit (50)*	SP54104
IndiSpin Pathogen Kit (250) *formerly QIAamp cador Pathogen Mini Kit (250)*	SP54106
intype IC-DNA	IC289980
intype IC-RNA	IC289970

INDICAL offers a broad range of ready-to-use pathogen specific ELISA kits, qPCR/ RT-qPCR assays and reagents.

To optimize your workflow, and to handle your sample and throughput needs, INDICAL additionally offers instruments and kits for the efficient extraction of nucleic acids from a variety of sample types.

Visit **www.indical.com** for more information about bactotype, cador, cattletype, flocktype, IndiMag, IndiSpin, intype, pigtype and virotype products.

For up-to-date licensing information and product-specific disclaimers, see the respective INDICAL product handbook or user manual.

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