

IndiMag[®] Pathogen Kit Handbook

For automated purification of viral RNA and DNA and bacterial DNA from animal samples using IndiMag 48, KingFisher[™] Flex, BioSprint[®] 96 or equivalent workstation



IndiMag Pathogen Kit w/o plastics (cat no SP947257),
formerly MagAttract[®] 96 cador[®] Pathogen Kit w/o Plastics (384)



IndiMag Pathogen Kit (cat no SP947457),
formerly MagAttract 96 cador Pathogen Kit (384)



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Kit contents

IndiMag Pathogen Kit	w/o plastics	
Cat. no.	SP947257	SP947457
Number of preps	384	384
Buffer VXL ¹	2 x 30 ml	2 x 30 ml
Buffer ACB ^{1,2}	2 x 60 ml	2 x 60 ml
Proteinase K	2 x 6 ml	2 x 6 ml
Carrier RNA (poly A)	2 x 310 µg	2 x 310 µg
MagAttract Suspension G ³	1 x 13 ml	1 x 13 ml
Buffer AW1 (concentrate) ^{1,4}	2 x 75.5 ml	2 x 75.5 ml
Buffer AW2 (concentrate) ⁴	2 x 54 ml	2 x 54 ml
Buffer AVE ³	1 x 125 ml	1 x 125 ml
Large 96-Rod Cover	-	4
S-Block	-	20
96-Well Microplate MP	-	4
Quick-Start Protocol (PCard)	2	2

1 CAUTION: Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 8 for safety information.

2 Before using for the first time, add isopropanol as indicated on the bottle to obtain a working solution.

3 CAUTION: Contains sodium azide.

4 Before using for the first time, add ethanol (96-100%) as indicated on the bottle to obtain a working solution.

Suitable workstations and protocols

Workstations

The following workstations are suitable, but are not limited to, when using the IndiMag Pathogen Kit (SP947457):

- KingFisher Flex
- KingFisher 96
- BioSprint 96
- MagMax™ Express 96

The following workstations are suitable, but are not limited to, when using the IndiMag Pathogen Kit w/o plastics (SP947257):

- IndiMag 48
- KingFisher
- KingFisher mL
- KingFisher Duo
- BioSprint 15
- MagMax Express

Protocols

Use the MagAttract 96 cadour Pathogen Kit Instrument Protocol (v2). Table 1 on page 6 gives an overview of script names depending on respective devices and used software systems.

Please note that the IndiMag 48 has the “Pathogen” protocol preinstalled.

Table 1: Script names depending on devices and software systems used.

Device	Software	Script name
BioSprint 96	BioSprint	BS96 cador v2.kf2
BioSprint 15	BioSprint	BS15 cador.kf2
KingFisher Duo	BindIt	KF_Duo_96_cador.bdz
KingFisher Flex	BindIt	KF_Flex_cador_v2.bdz
KingFisher 96	BindIt	KF_96_cador_v2.bdz
KingFisher 96	KingFisher	KF96 cador v2.kf2
KingFisher mL	KingFisher	KFmL cador.kf2

For further information or technical questions, please contact our INDICAL Support Team under **support@indical.com**.

Storage

All buffers and reagents are stable until the expiration date on the kit box at room temperature (15-25°C) 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 mixture, as described in “Preparing reagents”, on page 22. Carrier RNA/Buffer AVE/Buffer VXL mix solution should be prepared fresh. 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 IndiMag Pathogen Kit is intended for the automated 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 using the IndiMag 48, KingFisher Flex, Biosprint 96 or equivalent workstation.

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 IndiMag Pathogen Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

Magnetic bead technology enables purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities. The purified nucleic acids are ready for use in downstream applications, such as amplification or other enzymatic reactions.

The IndiMag Pathogen Kit enables the rapid purification of viral RNA and DNA, as well as bacterial DNA, from a broad range of animal sample types (see Table 2 on page 15) using the IndiMag 48, KingFisher Flex, BioSprint 96 or equivalent workstation (see “Starting material” on page 18). However, specific combinations of sample types and pathogens should be validated by the user.

Principle and procedure

The IndiMag Pathogen Kit uses MagAttract magnetic-particle technology for nucleic acid purification. This technology combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles (Figure 1, page 11).

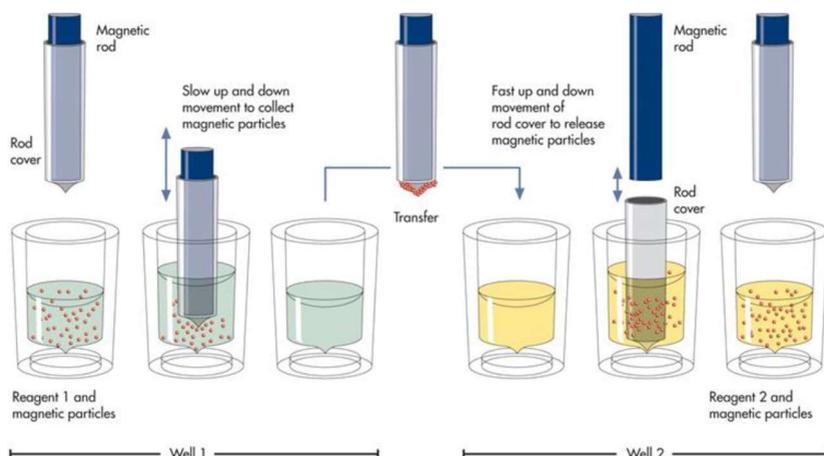


Figure 1. Schematic of the magnetic bead principle. The workstation processes a sample containing magnetic particles, as follows: Step 1) A magnetic rod, protected by a rod cover, enters a well (see well 1 in the figure) containing the sample and attracts the magnetic particles. Step 2) The magnetic rod cover is positioned above another well (see well 2 in the figure) and the magnetic particles are released. Steps 1 and 2 are repeated several times during sample processing.

The purification procedure is designed to ensure convenient, reproducible handling of potentially infectious samples (Figure 2, page 12).

Depending on the starting material, samples can be lysed in a single step in the presence of chaotropic salts and Proteinase K, releasing nucleic acids to bind to the silica surface of the MagAttract magnetic

particles. DNA and RNA bound to the magnetic particles are then efficiently washed, followed by an air drying step. High-quality nucleic acids are eluted in Buffer AVE. Nucleic acid yields depend on sample type and sample storage.

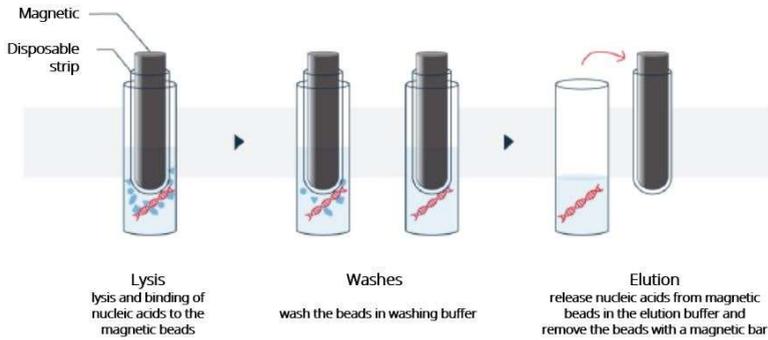


Figure 2. Schematic description of protocol steps

Description of protocols

There are two protocols in this handbook. In the first protocol, samples undergo nucleic acid purification on the IndiMag 48 workstation, whereas in the second protocol, samples are purified on KingFisher Flex, BioSprint 96 or equivalent workstations.

For samples that require a pretreatment prior to nucleic acid purification, Table 2 on page 15 provides an overview of which pretreatment protocols are suited to different starting material and pathogen combinations.

Sample purification time is approximately 34 minutes, not including upfront handling steps for prefilling S-Blocks or 96-well microplates. 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 8.

Nucleic acid purification protocol

The “Purification of Pathogen Nucleic acids from Fluid Samples” protocol (page 25) 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*

* 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 2 on page 15 summarizes the pretreatments and their applications.

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

Table 2: Pretreatment protocols for fluid and tissue samples

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
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
	<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> (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, 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.

- IndiMag 48 workstation, KingFisher Flex, BioSprint 96 or equivalent
 - If applicable: Magnetic head for use with Large 96-Rod Covers
 - 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
 - Soft cloth or tissue and 70% ethanol or other disinfectant to clean the used worktable
- Note:** please read the respective user manual for cleaning and maintenance the extraction device

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

Important notes

Starting material

The protocols in this handbook are optimized for purification of viral and bacterial nucleic acids, from easy-to-lyse sample types of low to moderate complexity. The IndiMag Pathogen protocol includes a special step that combines efficient lysis and binding in a single step, enabling quick, straight-forward sample processing. For sample types of higher complexity, such as tissue, feces and certain difficult-to-lyse pathogens, such as Gram-positive bacteria, specialized disruption and/or lysis pretreatments may be necessary. The user should determine appropriate pretreatments in advance, for such materials. General information about recommended sample types is given in the following sections. For further information, contact INDICAL support at **support@indical.com**.

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 2-8°C for up to 6 hours. For longer storage, we recommend freezing aliquots at -30 to -15°C or at -70°C.

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.

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

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.

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. 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.

Note: Solid pieces remaining in the homogenate may aggregate with the MagAttract magnetic particles, which could decrease nucleic acid yield.

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 IndiMag 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 magnetic particles, 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.

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 IndiMag 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 3 on page 26. 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.

Proteinase K

The IndiMag 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. Tick the check box on the bottle label to indicate that Isopropanol has been added. Mix well after adding Isopropanol.

MagAttract Suspension G

Shake the bottle containing MagAttract Suspension G and vortex for 3 minutes (before first use) or 1 minute (before subsequent uses) to ensure that the magnetic silica particles are fully resuspended.

Buffer AW1

Buffer AW1 is supplied as a concentrate. Before using for the first time, add Ethanol (96-100%) as indicated on the bottle. 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.

Buffer AW2

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

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 using the IndiMag 48, KingFisher Flex, BioSprint 96 or equivalent workstation and the IndiMag Pathogen Kit.

Important points before starting

- Ensure that you are familiar with the correct operation of the workstation. Refer to the respective user manual for operating instructions.
- Before beginning the procedure, read “Important notes” (page 18).
- Check that Buffer ACB, Buffer AW1, Buffer AW2, and Carrier RNA have been prepared according to the instructions in “Preparing reagents” (page 22).
- 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.
- If using IndiMag Pathogen Kit (SP947457): The 96-rod covers are supplied as packets of 2. When using a new packet of 2, store the second 96-rod cover on another S-block or plate. Care should be taken to not bend the 96-rod covers.

Things to do before starting

- 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.
- Prepare the Buffer VXL mixture according to Table 3 on page 26, for use in step 3 of the procedure. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 minutes before using for the first time or 1 minute before subsequent uses.

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

Table 3: Buffer VXL mixture preparation

Reagent	Number of samples *		
	1	48	96
Buffer VXL	100 µl	4.8 ml	9.6 ml
Buffer ACB	400 µl	19.2 ml	38.4 ml
MagAttract Suspension G	25 µl	1.2 ml	2.4 ml
Carrier RNA (1 µg/µl)	1 µl	48 µl	96 µl

* The volume prepared is 105% of the required volume to compensate for pipetting error and possible evaporation. Excess buffer should be discarded.

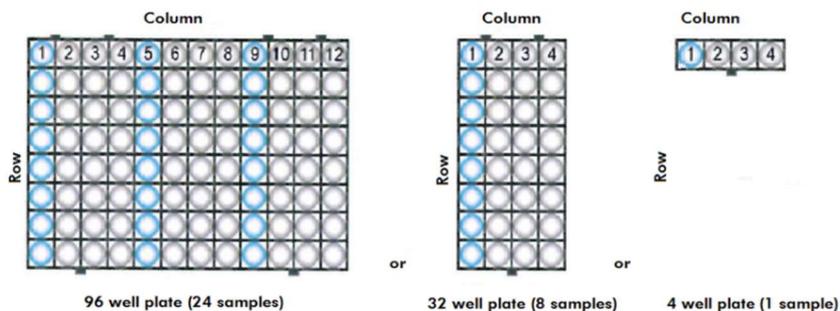
Procedure for use with the IndiMag 48

1. Label and prepare 4/32/96 well plates (columns 2-4) according to Table 3.

Table 3: Instrument setup and reagent volumes

Column	Item to add	Volume per well
1	Lysate	Lysate*
2	Wash 1	Buffer AW1
3	Wash 2	Buffer AW2
4	Elution	Buffer AVE

* Includes 20 µl Proteinase K, 200 µl sample and 500 µl Buffer VXL mixture



2. Ensure to have prepared enough Buffer VXL mixture according to Table 3, page 26.
3. Pipet 20 µl Proteinase K into the bottom of the first column and add 200 µl sample.

Note: If your sample volume is less than 200 µl, bring it to 200 µl by adding PBS.

4. Mix Buffer VXL mixture thoroughly for 30 s and add 500 µl Buffer VXL mixture to each sample in the deep well plate.

5. Immediately load the prepared plates onto the IndiMag 48, load the magnet rod cover strips on correct positions and start the appropriate protocol.

Note: the “Pathogen” protocol is preinstalled on the IndiMag 48.

Procedure for use with magnetic particle processors (e.g., KingFisher Flex, BioSprint 96 or equivalent)

1. Label and prepare 4 x 96-well deep well plates (S-Block) and 1 x 96-well microplate (slots 2-6) according to Table 4.

Table 4: Instrument setup and reagent volumes

Slot	Loading message	Format	Item to add	Volume per well
6	Load Rod Cover	96-well deep well plate	Cover for 96 tip comb	—
5	Load Elution	96-well microplate	Buffer AVE	100 µl
4	Load Wash 3	96-well deep well plate	Ethanol (96-100%)	750 µl
3	Load Wash 2	96-well deep well plate	Buffer AW2	700 µl
2	Load Wash 1	96-well deep well plate	Buffer AW1	700 µl
1	Load Lysate	96-well deep well plate	Lysate*	720 µl

* Includes 20 µl Proteinase K, 200 µl sample and 500 µl Buffer VXL mixture

2. Ensure to have prepared enough Buffer VXL mixture according to Table 3, page 26.
3. Pipet 20 µl Proteinase K into the bottom of a new well of the 96-well deep plate or S-Block and add 200 µl sample.

Note: If your sample volume is less than 200 µl, bring it to 200 µl by adding PBS.

4. Mix Buffer VXL mixture thoroughly for 30 s and add 500 μ l Buffer VXL mixture to each sample in the 96-well deep well plate.
5. Immediately load the prepared plates onto the processor and start the respective protocol.

Note: Table 1 on page 6 gives an overview of devices and their corresponding script names.

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	
Low yield of DNA and RNA	
1 MagAttract Suspension G not completely resuspended	Ensure that the MagAttract Suspension G is fully resuspended before adding to the Buffer VXL mixture. Vortex for at least 3 min before the first use, and for 1 min before subsequent uses.
2 Buffer VXL mixture prepared incorrectly	Ensure that Buffer VXL mixture was prepared with the correct volumes of additional reagents, as indicated on the buffer bottle. Repeat the DNA purification procedure with new samples.
3 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.
4 Reagents loaded onto worktable in wrong order	Ensure that all reagents are loaded onto the BioSprint 96 worktable in the correct order. Repeat the purification protocol with new samples.

5	Insufficient sample lysis	<p>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 7).</p> <p>For some DNA viruses and bacteria, heated lysis may improve lysis efficiency. For this purpose, an off-board-lysis protocol is available. Please contact INDICAL Support at support@indical.com.</p>
6	Carrier RNA not added to Buffer VXL or degraded Carrier RNA	Please refer to the recommendations for preparation, storage, and addition of carrier RNA.
7	Buffer VXL/Proteinase K/Carrier RNA mixture mixed insufficiently	Mix well by pipetting with a large pipette.
8	RNase contamination in Buffer AVE	Take care not to introduce RNases, which can degrade viral RNA. This may occur if tubes containing Buffer AVE are opened repeatedly. 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 (15-25°C) 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 “Low yield of viral DNA and RNA” (above) for possible reasons. Increase the amount of eluate added to the reaction, if possible.
2	Carryover of magnetic particles	Carryover of magnetic particles in eluates does not affect most downstream applications. Magnetic-particle carryover can be minimized by placing the microplate containing eluates in a suitable magnet (e.g., 96-Well Magnet Type A or 12-Tube Magnet for 1 min, and transferring the eluates to a clean microplate. If a suitable magnet is not available, centrifuge the microplate containing eluates at full

	speed for 1 min to pellet any remaining magnetic particles, and transfer the supernatants to a clean microplate.
3 Excessive eluate in the amplification reaction	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction, accordingly.
4 Degraded RNA	RNA may have been degraded by RNases in the original samples. Ensure that the samples are processed immediately after collection or recovery from storage. Repeat the purification protocol with new samples.
5 Nucleic acids in samples already degraded prior to purification	Samples were freeze-thawed more than once or stored at room temperature (15–25°C) for too long. Always use fresh samples or samples thawed only once. Repeat the purification protocol with new samples.
6 Carrier RNA not added to Buffer VXL mixture	Reconstitute Carrier RNA in Buffer AVE and mix with an appropriate volume of Buffer AVE, as described in “Using Carrier RNA and internal controls” (page 21). Repeat the purification protocol with new samples.
7 Too much or too little 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 mixture, accordingly.
8 Degraded Carrier RNA	Carrier RNA reconstituted in Buffer AVE was not stored at -30°C to -15°C or underwent multiple freeze-thaw cycles. Prepare a new tube of Carrier RNA dissolved in Buffer AVE and store appropriately. Repeat the purification procedure with new samples.
9 PCR inhibition	Some sample types (e.g., animal whole blood and feces) may contain high amounts of PCR inhibiting substances. Removal of inhibitors may not be complete without special treatment. Reduce the amount of sample input or/and the amount of eluate added to the amplification reaction.

Precipitate in buffers	
1	<p>Precipitate in Buffer VXL or Buffer ACB</p> <p>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.</p>
2	<p>Precipitate in sample-Buffer VXL mixture</p> <p>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.</p>

Order information

Product name	Cat. no.
IndiMag Pathogen Kit (384) <i>formerly MagAttract 96 cador Pathogen Kit (384)</i>	SP947457
IndiMag Pathogen Kit w/o plastics (384) <i>formerly MagAttract 96 cador Pathogen Kit w/o Plastics (384)</i>	SP947257
IndiMag 48	IN-942048
IndiMag 48 PW 1-Sample Block (368)	PW940123
IndiMag 48 PW 8-Sample Block (560)	PW940166
IndiMag 48 PW 24-Sample Block (672)	PW940187
IndiMag 48 PW Rod cover (672)	PW940237
intype IC-DNA	IC289980
intype IC-RNA	IC289970

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HB-1926-EN-004	October 2018	INDICAL design