September 2021 | EN

IndiMag® Pathogen Cartridge Handbook

For automated purification of viral RNA and DNA and bacterial DNA from animal samples using Magnetic Particle Processors

REF 2 x 24 blocks (cat no SP947654P224) for use with IndiMag 48/s

REF 6 x 8 blocks (cat no SP947654P608) for use with IndiMag 48/s

REF 1 x 96 blocks (cat no SP947855P196) for use with KingFisher™ Flex, BioSprint® 96 or equivalent workstation

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INDICAL BIOSCIENCE

Contents

Kit contents

IndiMag Pathogen Cartridge **Cat. no.** **Number of preps**	**SP947654P22448**	**SP947654P60848**	**SP947855P19696**
Cartridge [1]	2 x 24	6 x 8	5 x 96 [3]
Lysis Buffer [2]	1 x 24 ml	1 x 24 ml	1 x 48 ml
Rod Cover	6 strips	6 strips	1 plate
Quick-Start Protocol (PCard)	1	1	1

1 CAUTION: Contains a chaotropic salt, ethanol, and sodium azide. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 8 for safety information.
2 CAUTION: Contains a chaotropic salt and isopropanol. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 8 for safety information.
3 1 plate of each: Lysate, Wash Buffer 1, Wash Buffer 2, Wash Buffer 3, Elution

Suitable workstations and protocols

Workstations

The following workstations are suitable when using the IndiMag Pathogen Cartridge:

For use of IndiMag Pathogen IM48 Cartridge

- IndiMag 48
- IndiMag 48s

For use of IndiMag Pathogen KF96 Cartridge

- KingFisher Flex
- KingFisher 96
- BioSprint 96

Protocols

Please note that the IndiMag 48 and IndiMag 48s are provided with the “Pathogen” protocol being preinstalled.

When using a KingFisher Flex or alternative device, use the appropriate script listed in Table 1 on page 6.

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

Device	Software	Script name
KingFisher Flex	BindIt	IndiMag_C_Pathogen_KF_Flex.bdz
KingFisher 96	BindIt	IndiMag_C_Pathogen_KF96.bdz
KingFisher 96	KingFisher	IndiMag_C_Pathogen_KF96.kf2
BioSprint 96	BioSprint	IndiMag_C_Pathogen_BS96.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 (15-25ºC) without affecting performance.

Intended use

The IndiMag Pathogen Cartridge 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 a magnetic particle processor, such as the IndiMag 48/s, KingFisher Flex 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.

The IndiMag Pathogen Cartridge and Lysis Buffer contain guanidine hydrochloride and 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 Cartridge 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 Cartridge 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 14) using a magnetic particle processor, such as the IndiMag 48/s, KingFisher Flex or equivalent workstation (see “Starting material” on page 17). However, specific combinations of sample types and pathogens should be validated by the user.

Principle and procedure

The IndiMag Pathogen Cartridge 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).

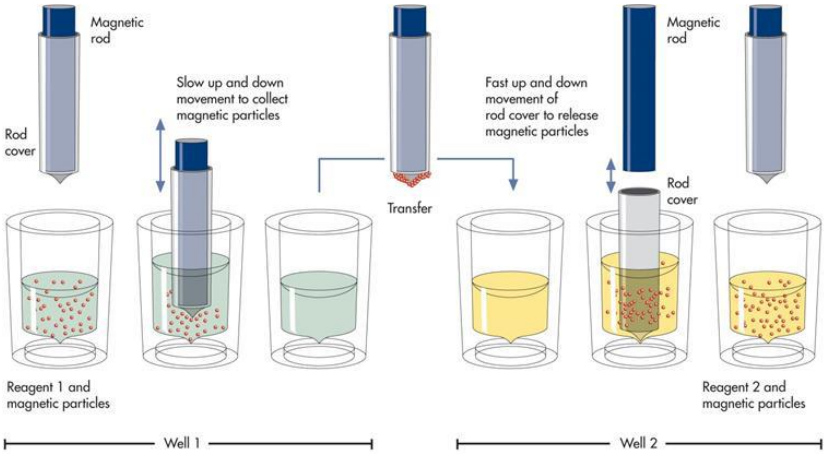

Figure 1. Schematic of the magnetic bead principle. The workstation processes a sample containing magnetic particles, as follows: Step 1) A magnetic rod, protected by a rod cover, enters a well (see well 1 in the figure) containing the sample and attracts the magnetic particles. Step 2) The magnetic 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.

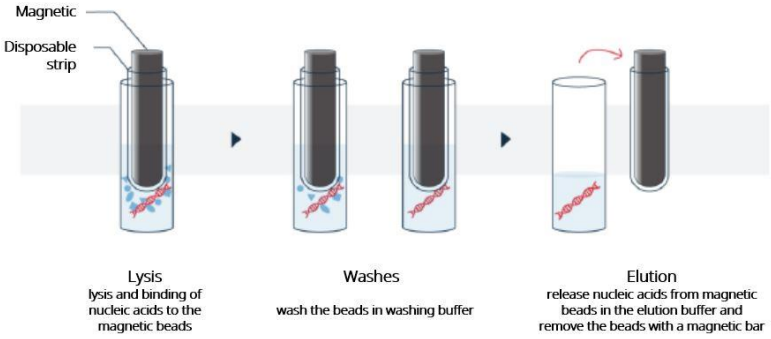

Figure 2. Schematic description of protocol steps

Nucleic acid purification protocol

The “Purification of Pathogen Nucleic acids from Fluid Samples” protocol (page 22) 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*

For samples that require a pretreatment prior to nucleic acid purification, Table 2 on page 14 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 centrifugation and pipetting samples and Lysis Buffer.

* 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 14 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**
High-volume cell-free fluids	Easy-to-lyse bacteria	**Pretreatment B3** for easy-to-lyse bacteria in high volume cell-free fluids	**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, use Pretreatment B1.

For further information on Pretreatments, please 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/ IndiMag 48s, KingFisher Flex or equivalent workstation
- Pipettors and disposable pipette tips with aerosol barriers (20–1000 µl)
- Multichannel pipettor and disposable 1000 µl pipette tips with aerosol barriers
- Multidispenser
- Phosphate-buffered saline (PBS), may be required for diluting samples
- Vortexer
- Centrifuge to spin down cartridges prior to use
- 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

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 2-8ºC 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.

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 Cartridge

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

The ready-to-use Lysis Buffer contains Carrier RNA.
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.

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 Cartridge 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 the Lysis Buffer 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.

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 a magnetic particle processor, such as the IndiMag 48/ IndiMag 48s or a KingFisher Flex and the compatible IndiMag Pathogen Cartridge.

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 17).

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.
- Depending on the kit being used, invert the IndiMag Pathogen IM48 Cartridge or “Wash 1” of IndiMag Pathogen KF96 Cartridge several times prior use, until the beads appear free in solution. Centrifuge the cartridge at room temperature (15-25°C) for 1 minute at 500 x *g*.

  Note: Centrifugation of IndiMag Pathogen IM48 cartridges require an IndiMag specific centrifugation adapter.

  Important: Do not add the Lysis Buffer directly to the first step column! This can cause clogs or precipitates. Follow the procedure

as described below (pipetting samples into the wells, followed by the Lysis Buffer).

Procedure for use with IndiMag 48 or IndiMag 48s

1. Invert the cartridge several times prior use, until the beads appear free in solution.
2. Centrifuge the cartridge in the IndiMag specific centrifugation adapter at room temperature (15-25°C) for 1 min at 500 x *g*.
3. Carefully peel off the foil covering the cartridge.
4. Pipet 200 µl sample into the bottom of the first column (marked in blue in Fig. 3).

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

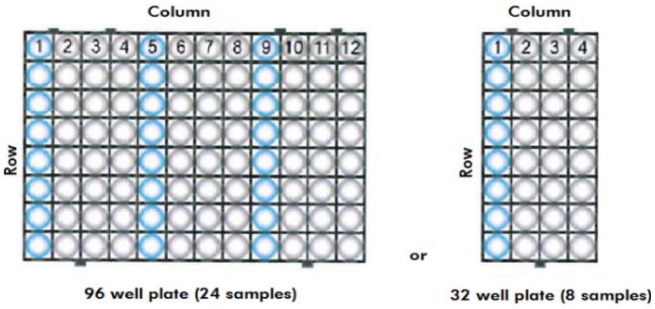

Figure 3. Types of cartridges and set up

5. Add 500 µl Lysis Buffer to each sample in the first column (marked in blue in Fig. 3).
6. Immediately load the prepared plates onto the IndiMag 48 or IndiMag 48s, load the magnet rod cover strips on correct positions and start the appropriate protocol.

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

Procedure for use with KingFisher Flex (or similar)

1. Invert the IndiMag Pathogen KF96 Cartridge “Wash 1” (②) several times prior use, until the beads appear free in solution.
2. Centrifuge the IndiMag Pathogen KF96 cartridges (①-⑤) in a 96-well-deep-well plate centrifugation adapter at room temperature (15-25°C) for 1 min at 500 x *g*.
3. Carefully peel off the foil covering the IndiMag Pathogen KF96 cartridge “Lysate” (①).
4. Pipet 200 µl sample into the bottom of the IndiMag Pathogen KF96 cartridge “Lysate” (①).

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

Table 3: Instrument setup KingFisher Flex or alternative

Slot	Loading message	Format	Item to add
6	Load Rod Cover	96-well deep well plate	Rod Cover
5	Load Elution	96-well microplate	Elution
4	Load Wash 3	96-well deep well plate	Wash 3
3	Load Wash 2	96-well deep well plate	Wash 2
2	Load Wash 1	96-well deep well plate	Wash 1
1	Load Lysate	96-well deep well plate	Lysate*

* Includes 200 µl sample and 500 µl Lysis Buffer

5. Add 500 µl Lysis Buffer to each sample in the IndiMag Pathogen KF96 cartridge “Lysate” (①).
6. Carefully peel off the foil covering the remaining IndiMag Pathogen KF96 cartridges (②-⑤).
7. Immediately load the prepared plates onto the processor, load the magnet rod cover plate and start the appropriate protocol.

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	Insufficient sample lysis	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**.
2	RNase contamination in Elution Buffer	Take care not to introduce RNases, which can degrade viral RNA. In case of RNase contamination, repeat the purification procedure with new samples.
3	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 “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 (15–25ºC) for too long. Always use fresh samples or samples thawed only once. Repeat the purification protocol with new samples.
6	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.

Order information

Product name	Cat. no.
IndiMag Pathogen IM48 Cartridge (2 x 24)	SP947654P224
IndiMag Pathogen IM48 Cartridge (6 x 8)	SP947654P608
IndiMag Pathogen KF96 Cartridge (1 x 96)	SP947855P196
IndiMag 48s	IN943048
IndiMag 48 PW Rod cover (672)	PW940237
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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Change index

Handbook	Version	Change
HB-2552-EN-002	September 2021	Product Relaunch
HB-2552-EN-001	August 2020	Product launch

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