

IndiMag® Pathogen Cartridge Handbook

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



IndiMag Pathogen IM2 Cartridge (sets for 6x8 samples)
(cat no SP957654C608) for use with IndiMag 2



IndiMag Pathogen IM48 Cartridge (6 x 8 sample blocks)
(cat no SP947654P608) for use with IndiMag 2 and IndiMag 48/s



IndiMag Pathogen IM48 Cartridge (2 x 24 sample blocks)
(cat no SP947654P224) for use with IndiMag 48/s



IndiMag Pathogen KF96 Cartridge (sets for 1x96, 4x96 or 16x96 samples (cat no SP947855P196, SP947855P496, SP947855P1696) for use with KingFisher™ Flex, BioSprint® 96 or equivalent workstation



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Kit contents IndiMag Pathogen Cartridges

Cartridges for use with IndiMag devices

Cat. no.	SP957654C608	SP947654P224	SP947654P608
Number of preps	48	48	48
Cartridge ¹	6 sets x 8 ³	2 x 24	6 x 8
Lysis Buffer ²	1 x 24 ml	1 x 24 ml	1 x 24 ml
Rod Cover	6 strips	6 strips	6 strips
Quick-Start Protocol (PCard)	1	1	1

Cartridges for use with KingFisher 96 devices or equivalent

Cat. no.	SP947855P196	SP947855P496	SP947855P1696
Number of preps	1 x 96	4 x 96	16 x 96
Cartridge ¹	5 x 96 ⁴	20 x 96 ⁵	The product contains 4 kits SP947855P496 in one larger kit box
Lysis Buffer ²	1 x 48 ml	2 x 48 ml	
Rod Cover	1 plate	2 x 2 plates	
Quick-Start Protocol (PCard)	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 6 sets of 8-sample lysis strips, 8-sample wash blocks, 8-sample elution blocks

4 1 plate of each: Lysate, Wash Buffer 1, Wash Buffer 2, Wash Buffer 3, Elution

5 4 plates 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: **Note:** The IndiMag 48 and the IndiMag 48s are no longer available for purchase.

IndiMag Pathogen IM2 Cartridge (sets for 6x8 samples)
(SP957654C608)

- IndiMag 2

IndiMag Pathogen IM48 Cartridge (2 x 24 sample blocks)
(SP947654P224)

- IndiMag 48
- IndiMag 48s

IndiMag Pathogen IM48 Cartridge (6 x 8 blocks)
(SP947654P608)

- IndiMag 48
- IndiMag 48s
- IndiMag 2

IndiMag Pathogen KF96 Cartridge
(SP947855P196, SP947855P496, SP947855P1696)

- KingFisher 96 sample purification systems
- Allsheng 96 sample purification systems
- BioSprint 96
- Other 96 sample extraction instruments

Protocols

The IndiMag Pathogen scripts “Pathogen” are preinstalled for the IndiMag Pathogen IM48 Cartridge (6 x 8 blocks) SP947654P608 and (2 x 24 sample blocks) (SP947654P224) on the IndiMag devices.

Multiple IndiMag Pathogen scripts are preinstalled on the IndiMag 2.

- The IndiMag Pathogen -/Block/- protocol for using IndiMag Pathogen IM48 Cartridge (Cat.no. SP947654P608) and/or
- The IndiMag Pathogen Lysis/Block/Elution” protocol for using IndiMag Pathogen IM2 Cartridge (sets for 6x8 samples) (SP957654C608)

When using a KingFisher 96 sample purification systems or alternative device, use the appropriate script listed in Table 1.

Table 1: Device, software, and script names for the specific instruments.

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
KingFisher Apex	BindIx	IndiMag C Pathogen.kfx
BioSprint 96	BioSprint	IndiMag_C_Pathogen_BS96.kf2

Scripts for additional instruments, technical questions, or further questions, please contact our INDICAL Support Team at support@indical.com (global) or us_support@indical.com (Americas).

Storage

All buffers and reagents are stable until the expiration date on the kit box at room temperature (15-25°C) 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 2, IndiMag 48/s, KingFisher 96 sample purification systems or equivalent workstation.

For molecular biology applications.

Symbols



Legal manufacturer



Lot number



Use by date



Temperature limitations for storage



Handbook



Catalogue 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 at 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, IndiMag 2, KingFisher 96 sample purification systems 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 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).

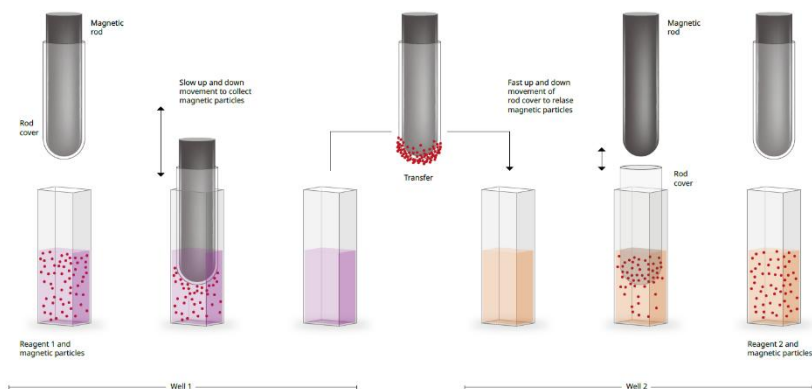


Figure 1. Schematic of the magnetic bead principle. The workstation processes a sample containing magnetic particles, as follows: Step 1) A magnetic rod, protected by a rod cover, enters a well (see well 1 in the figure) containing the sample and attracts the magnetic particles. Step 2) The magnetic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

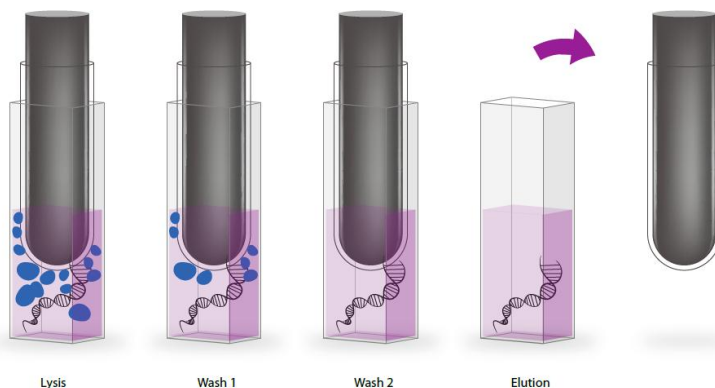


Figure 2. Schematic description of protocol steps

Nucleic acid purification protocol

The “Purification of Pathogen Nucleic acids from Fluid Samples” protocol (page 24) 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 (Global) or us_support@indical.com (Americas)).

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

Organic extraction of difficult biological fluids	Viral RNA and DNA, easy-to-lyse bacteria DNA	Pretreatment B4	HB-2642
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
Tissue	<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> (MAP) DNA	Pretreatment T-MAP (tissue)	HB-2644
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 (feces)	HB-2503
Filter paper cards		Pretreatment C1	HB-2520

Swabs (tracheal, oropharyngeal, blood)		Pretreatment S1	HB-2516
Oral fluid (chew ropes)	Viral RNA and DNA, bacterial DNA	Pretreatment O2	HB-2564
Semen (bulls)	SBV RNA	Pretreatment SE-SBV	HB-2640
Plants	Nucleic acids	Pretreatment P1	HB-2643
Hair (animals)	Nucleic acids	Pretreatment H1	HB-2664

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 (Global) or us_support@indical.com (Americas).

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 2, IndiMag 48/s, KingFisher 96 sample purification systems 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), 0.9 % NaCl solution, or nuclease-free water 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 (Global) or us_support@indical.com (Americas)

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 (Global) or us_support@indical.com (Americas) 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 titres, 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 nuclease-free water.

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 nuclease-free water.

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 regards 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 number 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 (IC289980) or intype IC-RNA (IC289970) 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 (Global) or us_support@indical.com (Americas)) 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 2, IndiMag 48/s, or a KingFisher 96 sample purification system 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 18).

Things to do before starting

- Thaw and equilibrate samples at room temperature (15-25°C).
- If working with insufficient volume of sample, add PBS, 0.9 % NaCl or nuclease-free water to achieve a total sample volume of 200 µl. Prior to use, invert the 32-well IndiMag Pathogen IM2 Cartridge, the IndiMag Pathogen IM48 Cartridge or the Wash 1-plate of IndiMag Pathogen KF96 Cartridge several times, until the magnetic beads are fully resuspended and freely dispersed in the 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 IndiMag Pathogen IM2 Cartridge for use with IndiMag 2

1. Invert the sealed 32-well blocks several times prior to use, until the beads appear free in solution.
2. Centrifuge the sealed 32-well block in the IndiMag-specific centrifugation adapter at room temperature (15-25°C) for 1 minute at 500 x *g*. For the lysis and elution strip, use the provided racks. Please ensure to follow standard practices for balancing centrifuges.

Note: The lysis (L) and elution strips (E) are sealed together. Carefully cut the required number of strips for your sample size before use.

3. Load the rod covers into correct positions.
4. Carefully peel off the foil covering the elution strip and the 32-well block and load the consumables into the IndiMag 2 according to Figure 3.
5. Carefully remove the foil covering the lysis strip, and pipet 200 µl sample into the bottom of the lysis strip.

Note: If working with insufficient volume of sample, add PBS, 0.9 % NaCl or nuclease-free water to achieve a total sample volume of 200 µl.

6. Add 500 µl Lysis Buffer to each sample in in the lysis strip.
7. Load the lysis strip into the IndiMag 2, according to Figure 3 (marked in purple) and start the “IndiMag Pathogen Lysis/Block/Elution” protocol.

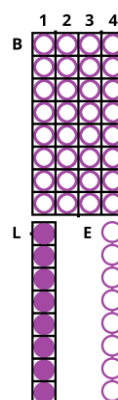


Figure 3:
Instrument Setup

Note: The “IndiMag Pathogen Lysis/Block/Elution” protocol is preinstalled on the IndiMag 2.

Procedure for IndiMag Pathogen IM48 Cartridge (6 x 8 only) for use with IndiMag 2

1. Invert the IndiMag Pathogen IM48 Cartridge several times prior to use, until the magnetic beads appear free in solution.
2. Centrifuge the IndiMag Pathogen IM48 Cartridge in the IndiMag-specific centrifugation adapter at room temperature (15-25°C) for 1 min at 500 x *g*.

3. Carefully remove the foil covering the Cartridge. Pipet 200 µl sample into the bottom of the first column of the cartridge according to Figure 4 (Block; marked in purple).

Note: If working with insufficient volume of sample, add PBS, 0.9 % NaCl or nuclease-free water to achieve a total sample volume of 200 µl.

4. Add 500 µl Lysis Buffer to each sample in column 1 of the Cartridges (B1; marked in purple).
5. Immediately load the cartridges into the IndiMag 2, load the rod covers into correct positions and start the IndiMag Pathogen -/Block/- protocol.

Note: The “IndiMag Pathogen -/Block/-” protocol is preinstalled on the IndiMag 2.



Figure 4:
Instrument
Setup

Procedure for IndiMag Pathogen IM48 Cartridges for use with IndiMag 48 or IndiMag 48s

1. Invert the Cartridges several times before use, until the beads appear free in solution.
2. Centrifuge the Cartridges in the IndiMag specific centrifugation adapter at room temperature (15-25°C) for 1 min at 500 x *g*.
3. Carefully remove the foil covering the Cartridges.
4. Pipet 200 µl sample into the bottom of the first column (marked in blue in Fig. 5).

Note: If working with insufficient volume of sample, add PBS, 0.9 % NaCl or nuclease-free water to achieve a total sample volume of 200 µl.

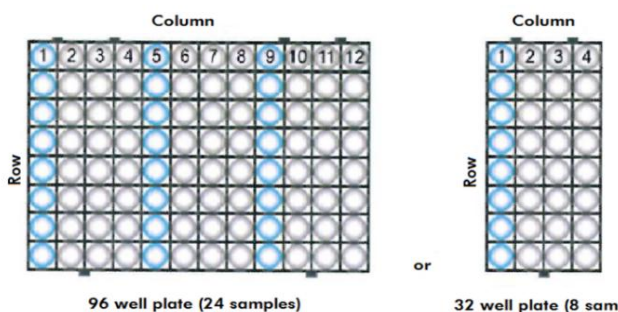


Figure 5. Types of cartridges and set up

5. Add 500 µl Lysis Buffer to each sample in the first column (marked in blue in Fig. 3).

6. Immediately load the prepared cartridges onto the IndiMag 48 or IndiMag 48s, load the rod covers into correct positions and start the appropriate protocol.

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

Procedure for IndiMag Pathogen KF96 Cartridges for use with KingFisher 96 sample purification system (or equivalent)

1. Invert the IndiMag Pathogen KF96 Cartridge sealed consumables several times before use. Ensure the beads appear free in solution in the "Wash 1" cartridge.
2. Centrifuge the IndiMag Pathogen KF96 cartridges in a 96-well-deep-well plate centrifugation adapter at room temperature (15-25°C) for 1 min at 500 x *g*.
3. Carefully remove the foil covering all the IndiMag Pathogen KF96 cartridges.
4. Load the IndiMag Pathogen KF96 cartridges slot 2-6 on the KingFisher, see Table 3.

Table 3: Instrument setup KingFisher 96 sample purification system (or equivalent).

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. Pipet 200 µl sample into the bottom of the IndiMag Pathogen KF96 cartridge "Lysate".

Note: If working with insufficient volume of sample, add PBS, 0.9 % NaCl or nuclease-free water to achieve a total sample volume of 200 µl.

6. Add 500 µl Lysis Buffer to each sample in the IndiMag Pathogen KF96 cartridge "Lysate".
7. Immediately load the "lysate" plate in the KingFisher 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 (Global) or us_support@indical.com (Americas).

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 (Global) or us_support@indical.com (Americas).
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 (15-25°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	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 2	IN950048
IndiMag Pathogen IM2 Cartridge (6 x 8)	SP957654C608
IndiMag Pathogen IM48 Cartridge (2 x 24)	SP947654P224
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Change index

Handbook	Version	Change
HB-2552-EN-003	May 2026	Implementation IndiMag Pathogen IM2 Cartridge
HB-2552-EN-002	September 2021	Product Relaunch



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