August 2025 | EN

IndiMag® Pathogen Kit Handbook

For automated purification of viral RNA and DNA, and bacterial DNA from animal samples using IndiMag 2, IndiMag 48/s, KingFisher™ Flex, BioSprint 96, or equivalent workstation

REF IndiMag Pathogen Kit w/o plastics (cat. no. SP947257)

REF IndiMag Pathogen Kit (cat. no. SP947457)

Manufactured by QIAGEN® GmbH for INDICAL BIOSCIENCE
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04103 Leipzig, Germany

INDICAL BIOSCIENCE

Contents

Kit contents

IndiMag Pathogen Kit Cat. no.	w/o plastics SP947257	 SP947457
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 10 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 IndiMag Pathogen Kit (SP947457) can be used with the following, non-exhaustive list of workstations:

- KingFisher Flex
- KingFisher 96
- BioSprint 96
- MagMax™ Express 96
- Allsheng Auto-Pure96

The IndiMag Pathogen Kit w/o plastics (SP947257) can be used with the following, non-exhaustive list of workstations:

- IndiMag 2
- IndiMag 48/s
- KingFisher mL
- KingFisher Duo
- KingFisher Apex
- BioSprint 15

Scripts

Use the “Pathogen instrument script”. Table 1 on page 7 gives an overview of script names depending on respective devices and used software systems. The scripts are available on the INDICAL website (https://www.indical.com) or through INDICAL’s technical support team.

In addition, the script “Pathogen version 2” with optimised elution step can be used. Table 2 on page 8 gives an overview of script names depending on respective devices and used software systems.

Please note that the “Pathogen” protocol is pre-installed on the IndiMag 2 and the IndiMag 48/s.

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

Device	Software	Script name (old)	Script name (new)
BioSprint 15	BioSprint	BS15 cador.kf2	IndiMag_Pathogen_BS15.kf2
BioSprint 96	BioSprint	BS96 cador v2.kf2	IndiMag_Pathogen_BS96.kf2
KingFisher mL	KingFisher	KFmL cador.kf2	IndiMag_Pathogen_KFmL.kf2
KingFisher 96	KingFisher	KF96 cador v2.kf2	IndiMag_Pathogen_KF96.kf2
KingFisher mL	BindIt	-	IndiMag_Pathogen_KF_mL.bdz
KingFisher 96	BindIt	KF_96_cador_v2.bdz	IndiMag_Pathogen_KF96.bdz
KingFisher Duo	BindIt	KF_Duo_96_cador.bdz	IndiMag_Pathogen_KF_Duo.bdz
KingFisher Flex	BindIt	KF_Flex_cador_v2.bdz	IndiMag_Pathogen_KF_Flex.bdz
King Fisher Apex	BindIx	-	IndiMag Pathogen.kfx
IDEAL 32	-	-	IndiMagPathogen.txt
IDEAL 96	-	-	Pathogen.txt
AutoPure 96	-	-	IndiMagPathogen.txt

Table 2: Script names depending on devices and software systems used (Pathogen version 2 with optimised elution step)

Device	Software	Script name (version 2)
BioSprint 15	BioSprint	IndiMag_Patho_B15_v2.kf2
BioSprint 96	BioSprint	IndiMag_Patho_B96_v2.kf2
KingFisher mL	KingFisher	IndiMag_Patho_Kml_v2.kf2
KingFisher 96	KingFisher	IndiMag_Patho_K96_v2.kf2
KingFisher mL	BindIt	IndiMag_Pathogen_KF_mL_v2.bdz
KingFisher 96	BindIt	IndiMag_Pathogen_KF96_v2.bdz
KingFisher Duo	BindIt	IndiMag_Pathogen_KF_Duo_v2.bdz
KingFisher Flex	BindIt	IndiMag_Pathogen_KF_Flex_v2.bdz
KingFisher Apex	BindIx	IndiMag Pathogen v2.kfx
AutoPure 96	-	IndiMagPathoV2.txt

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

Storage

All buffers and reagents are stable at room temperature (15-25ºC) without affecting performance until the expiration date on the kit box.

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 28. 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 three 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, feces, and tissue homogenate using the IndiMag 2, IndiMag 48/s, 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 to 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 sample types (see page 16) using the IndiMag 2, IndiMag 48/s, KingFisher Flex, BioSprint 96 or equivalent workstation (see “Starting material” on page 21). 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 13).

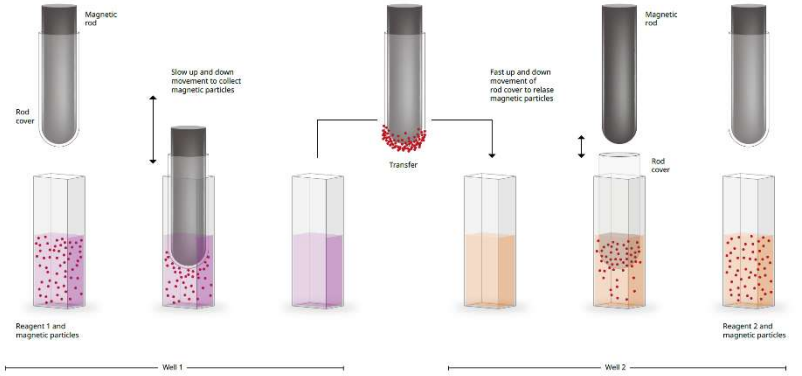
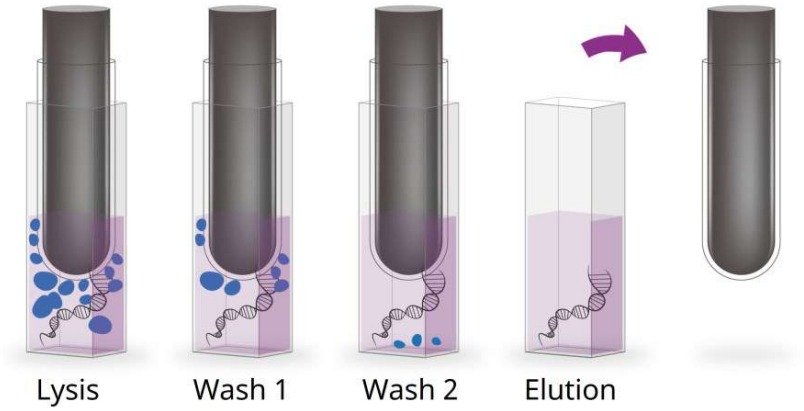


Figure 1. Schematic description of the magnetic bead principle. The workstation processes a sample containing magnetic particles, as follows: Step 1) A magnetic rod, protected by a rod cover, enters a well (see well 1 in the figure) containing the sample and attracts the magnetic particles. Step 2) The 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 14).

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 different procedures described in this handbook, describing nucleic acid purification run on various workstations, such as the IndiMag 2, IndiMag 48/s, KingFisher Flex, BioSprint 96 or equivalent workstations.

For samples that require a pretreatment prior to nucleic acid purification, Table 3 on page 17 provides an overview of which pretreatment protocols are suited to different starting material and pathogen combinations.

Sample purification time is approximately 34 min, 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 10.

Nucleic acid purification protocol

The “Purification of pathogen nucleic acids from fluid samples” protocol (page 31) 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
- Milk
- 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 3 on page 17 summarizes the pretreatments and their applications.

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

Table 3: 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
Cell-Free Fluids	DNA of easy-to-lyse bacteria	**Pretreatment B3** for easy-to-Lyse Bacteria in high volume cell-free fluids	HB-2549

Fluids containing a high amount of lipids and/or nucleases	Viral RNA and DNA, DNA of easy-to-lyse bacteria[1]	**Pretreatment B4** Organic extraction for difficult fluids	**HB-2642**
Filter paper cards	Pathogen nucleic acids	**Pretreatment C1**	**HB-2520**
Feces	Viral RNA and DANN	**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**
Oral fluid applications	Pathogen nucleic acids	**Pretreatment O2**	**HB-2564**
Semen	RNA from bull semen	**Pretreatment SE-SBV**	**HB-2640**
Swabs (tracheal, oropharyngeal, blood)	Pathogen nucleic acids	**Pretreatment S1**	**HB-2516**
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 (e.g., liver, spleen, kidney, lymph node)	*Mycobacterium avium* subsp. *paratuberculosis* (MAP) DNA	**Pretreatment T-MAP**	**HB-2644**
Plants	Pathogen nucleic acids	**Pretreatment P1**	**HB-2643**

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, visit **www.indical.com/handbooks** or 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 2 or IndiMag 48/s workstation, KingFisher Flex, BioSprint 96 or equivalent
- If applicable: Magnetic head for use with Large 96-Rod Covers
- Liquid handling equipment and consumables, incl. (multichannel) pipet and pipet tips (aerosol-resistant pipet tips with filters are recommended to prevent cross-contamination)
- Multidispenser and corresponding tips
- Vortexer
- Ethanol (96-100 %)*
- Isopropanol
- Optional: Phosphate-buffered saline (PBS), 0.9 % NaCl, or nuclease-free water may be required for diluting samples
- 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 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, if 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 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 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 fluids, 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, 0.9 % NaCl, or water.

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. See Pretreatment B2 for extraction of DNA from difficult-to-lyse bacteria. See Pretreatment B3 for extraction of bacterial DNA of easy-to-lyse bacteria from high volume of cell-free fluids.

Animal tissues

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

Different tissue types can vary widely regarding 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 the 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 4 on page 32. 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 three 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 VXL

Buffer VXL is a ready-to-use lysis buffer and can be stored at room temperature.

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 min (before first use) or 1 min (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. Reconstituted Buffer AW2 can be stored at room temperature (15-25°C) for up to 1 year. 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 2, IndiMag 48/s, 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 21).
- Check that Buffer ACB, Buffer AW1, Buffer AW2, and Carrier RNA have been prepared according to the instructions in “Preparing reagents” (page 28).
- Check that Buffer VXL or Buffer ACB do not contain a white precipitate. If necessary, incubate Buffer VXL or Buffer ACB for 30 min 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).
- Prepare the Buffer VXL mixture according to Table 4 on page 32, for use in step 4 of the procedure. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 min before using for the first time or 1 min before subsequent uses.

  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 mixture).

Table 4: 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 2 - using lysis strips, 32-well blocks and elution strips

1. Label and prepare 32-well blocks (B; columns 1-4), lysis strips (L) and elution strips (E) according to Table 5 and Figure 3.

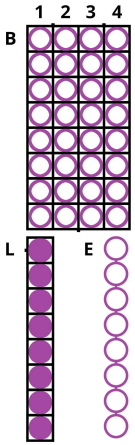
Table 5 and Figure 3 (right): Instrument setup and reagent volumes

Lysis strip (L)	Item to add	Volume per well
L Lysate	Lysate*	720 µl*

Column (B)	Item to add	Volume per well
1 Wash 1	Buffer AW 1	700 µl
2 Wash 2	Buffer AW 2	700 µl
3 Wash 3	Ethanol (96 – 100 %)	750 µl
4 *empty*	-	-

Elution strip (E)	Item to add	Volume per well
E Elution	Buffer AVE	100 µl

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



2. Prepare Buffer VXL mixture according to Table 6.

Table 6: Buffer VXL mixture preparation

Reagent	1 reaction	8 reactions	24 reactions	48 reactions
Buffer VXL	100 µl	800 µl	2.4 ml	4.8 ml
Buffer ACB	400 µl	3.2 ml	9.6 ml	19.2 ml
MagAttract Suspension G	25 µl	200 µl	0.6 ml	1.2 ml
Carrier RNA (1 µg/µl)	1 µl	8 µl	24 µl	48 µl

3. Pipet 20 µl Proteinase K into the bottom of the lysis strips and add 200 µl sample according to Table 5 and Figure 3 (L; 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. Mix Buffer VXL mixture thoroughly for 30 sec and add 500 µl Buffer VXL mixture to each sample (L; marked in purple).
5. Immediately load the prepared 32-well blocks and strips into the IndiMag 2, load the rod covers into correct positions and start the appropriate protocol.

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

Procedure for use with the IndiMag 2 - using 32-well blocks only

1. Label and prepare 32-well blocks (B; columns 2-4) according to Table 7 and Figure 4.

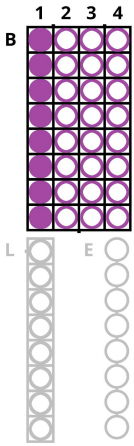


Table 7 and Figure 4 (right): Instrument setup and reagent volumes

Column (B)	Item to add	Volume per well
1 Lysate	Lysate*	720 µl
2 Wash 1	Buffer AW 1	700 µl
3 Wash 2	Buffer AW 2	700 µl
4 Elution	Buffer AVE	100 µl

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

2. Prepare Buffer VXL mixture according to Table 8.

Table 8: Buffer VXL mixture preparation

	Reactions			
Reagent	1	8	24	48
Buffer VXL	100 µl	800 µl	2.4 ml	4.8 ml
Buffer ACB	400 µl	3.2 ml	9.6 ml	19.2 ml
MagAttract Suspension G	25 µl	200 µl	0.6 ml	1.2 ml
Carrier RNA (1 µg/µl)	1 µl	8 µl	24 µl	48 µl

3. Pipet 20 µl Proteinase K into the bottom of the first column of the 32-well-blocks and add 200 µl sample according to Table 7 and Figure 4 (B1; 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. Mix Buffer VXL mixture thoroughly for 30 sec and add 500 µl Buffer VXL mixture to each sample in column 1 in the 32-well blocks (B1; marked in purple).
5. Immediately load the prepared 32-well blocks into the IndiMag 2, load the rod covers into correct positions and start the appropriate protocol.

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

Procedure for use with the IndiMag 2 - using lysis strips and 32-well blocks

1. Label and prepare 32-well blocks (B; columns 2-4), and lysis strips (L) according to Table 9 and Figure 5.

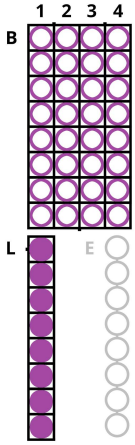


Table 9 and Figure 5 (right): Instrument setup and reagent volumes

Lysis strip (L)	Item to add	Volume per well
1 Lysate	Lysate*	720 µl

Column (B)	Item to add	Volume per well
1 Wash 1	Buffer AW 1	700 µl
2 Wash 2	Buffer AW 2	700 µl
3 Wash 3	Ethanol (96-100 %)	750 µl
4 Elution	Buffer AVE	100 µl

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

2. Prepare Buffer VXL mixture according to Table 10.

Table 10: Buffer VXL mixture preparation

	Reactions			
Reagent	1	8	24	48
Buffer VXL	100 µl	800 µl	2.4 ml	4.8 ml
Buffer ACB	400 µl	3.2 ml	9.6 ml	19.2 ml
MagAttract	25 µl	200 µl	0.6 ml	1.2 ml
Carrier RNA (1 µg/µl)	1 µl	8 µl	24 µl	48 µl

3. Pipet 20 µl Proteinase K into the bottom of the lysis strips and add 200 µl sample according to Table 9 and Figure 5 (L; 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. Mix Buffer VXL mixture thoroughly for 30 sec and add 500 µl Buffer VXL mixture to each sample in the lysis strips (L; marked in purple).
5. Immediately load the prepared 32-well blocks and lysis strips into the IndiMag 2, load the rod covers into correct positions and start the appropriate protocol.

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

Procedure for use with the IndiMag 2 - using 32-well blocks and elution strips

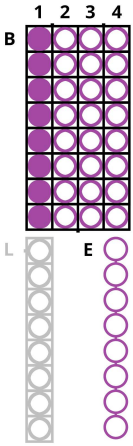
1. Label and prepare 32-well blocks (B; columns 2-4), and elution strips (E) according to Table 11 and Figure 6.

Table 11 and Figure 6 (right): Instrument setup and reagent volumes

Column (B)	Item to add	Volume per well
1 Lysate	Lysate*	720 µl
2 Wash 1	Buffer AW 1	700 µl
3 Wash 2	Buffer AW 2	700 µl
4 Wash 3	Ethanol (96-100 %)	750 µl

Elution strip (E)	Item to add	Volume per well
E Elution	Buffer AVE	100 µl

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



2. Prepare Buffer VXL mixture according to Table 12.

Table 12: Buffer VXL mixture preparation

	Reactions			
Reagent	1	8	24	48
Buffer VXL	100 µl	800 µl	2.4 ml	4.8 ml
Buffer ACB	400 µl	3.2 ml	9.6 ml	19.2 ml
MagAttract	25 µl	200 µl	0.6 ml	1.2 ml
Carrier RNA (1 µg/µl)	1 µl	8 µl	24 µl	48 µl

3. Pipet 20 µl Proteinase K into the bottom of the first column of the 32-well blocks and add 200 µl sample according to Table 11 and Figure 6 (B1; 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. Mix Buffer VXL mixture thoroughly for 30 sec and add 500 µl Buffer VXL mixture to each sample in the 32-well blocks B1; marked in purple).
5. Immediately load the prepared 32-well plates and elution strips into the IndiMag 2, load the rod covers into correct positions and start the appropriate protocol.

   Note: The “IndiMag Pathogen 0/Block/Elution” protocol is preinstalled on the IndiMag 2.

Procedure for use with the IndiMag 48/s

1. Label and prepare 4/32/96 well plates (columns 2-4; Figure 7) according to Table 13.

Table 13: Instrument setup and reagent volumes

Column		Item to add	Volume per well
1	Lysate	Lysate*	720 µl
2	Wash 1	Buffer AW1	700 µl
3	Wash 2	Buffer AW2	700 µl
4	Elution	Buffer AVE	100 µl

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

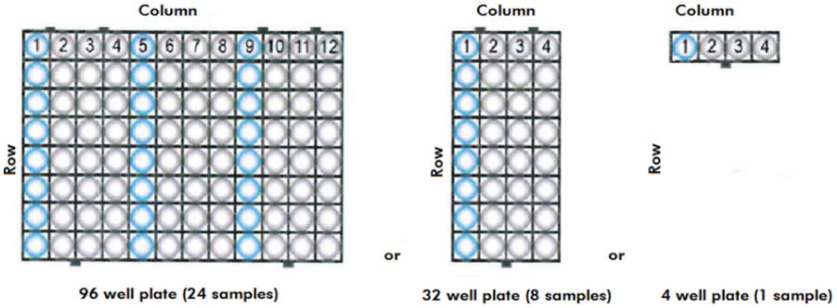


Figure 7. Instrument setup.

2. Ensure to have prepared enough Buffer VXL mixture according to Table 4, page 32.
3. Pipet 20 µl Proteinase K into the bottom of the first column and add 200 µl sample (see Figure 7).

   Note: If necessary, add PBS, 0.9 % NaCl, or water to achieve a total sample volume of 200 µl.

4. Mix Buffer VXL mixture thoroughly for 30 sec 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/s, load the magnet rod cover strips on correct positions and start the appropriate protocol.

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

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 according to Table 14 (slots 2-6).

Table 14: 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 4, page 32.
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 necessary, add PBS, 0.9 % NaCl, or water to achieve a total sample volume of 200 µl.

4. Mix Buffer VXL mixture thoroughly for 30 sec and add 500 µl 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 (page 7) and Table 2 (page 8) give an overview of devices and their corresponding script names.

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

1. Label and prepare one 96-well deep well plate (rows B-E) and one KingFisher Duo elution strip according to Table 15.

Table 15: Instrument setup and reagent volumes

Row	Format	Item to add	Volume per well
-	KingFisher Duo elution strip	Buffer AVE	100 µl
A	96-well deep well plate	Lysate*	720 µl
B	96-well deep well plate	Buffer AW1	700 µl
C	96-well deep well plate	Buffer AW2	700 µl
D	96-well deep well plate	Ethanol (96–100 %)	750 µl
E	96-well deep well plate	KingFisher Duo 12-tip comb	—

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

2. Prepare Buffer VXL mixture according to Table 16.

Table 16: Buffer VXL mixture preparation

Reagent	Number of samples *	
	1	12
Buffer VXL	100 µl	1.2 ml
Buffer ACB	400 µl	4.8 ml
MagAttract Suspension G	25 µl	0.3 ml
Carrier RNA (1 µg/µl)	1 µl	12 µl

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

3. Pipet 20 µl Proteinase K into the bottom of a new well of the 96-well deep well plate well and add 200 µl sample (row A) according to Table 15.

   Note: If necessary, add PBS, 0.9 % NaCl, or water to achieve a total sample volume of 200 µl.

4. Mix Buffer VXL mixture thoroughly for 30 sec and add 500 µl 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 appropriate script.

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	Buffers AW1, Buffer AW2, or Buffer ACB prepared incorrectly	Check that Buffer AW1 or Buffer AW2 concentrate was diluted with the correct volume of 96–100 % ethanol, and that Buffer ACB was diluted with the correct volume of isopropanol, 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 magnetic particle processor worktable in the correct order. Repeat the purification protocol with new samples.

5	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 9). 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.
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 mixture mixed insufficiently	Mix well by vortexing for at least 30 sec.
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 (15-25ºC) 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 transfer of 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	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 25). Repeat the purification protocol with new samples.
6	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.

7	Degraded Carrier RNA	Carrier RNA reconstituted in Buffer AVE was not stored at -30°C to -15ºC 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.
8	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	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.
IndiMag Pathogen Kit	SP947457
IndiMag Pathogen Kit w/o plastics	SP947257
IndiMag 2 (100~240 V)	IN950048
IndiMag 2 PW Lysis Strips (672)	PW950215
IndiMag 2 PW Lysis Rack (6)	PW950225
IndiMag 2 PW Elution Set (384)	PW950235
IndiMag 48s (100~240 V)	IN943048S
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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Notes

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Change index

Handbook	Version	Change
HB-1926-EN-005	August 2025	Implementation of IndiMag 2 and new Pretreatment Protocols; editorial changes
HB-1926-EN-004	October 2018	INDICAL design

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