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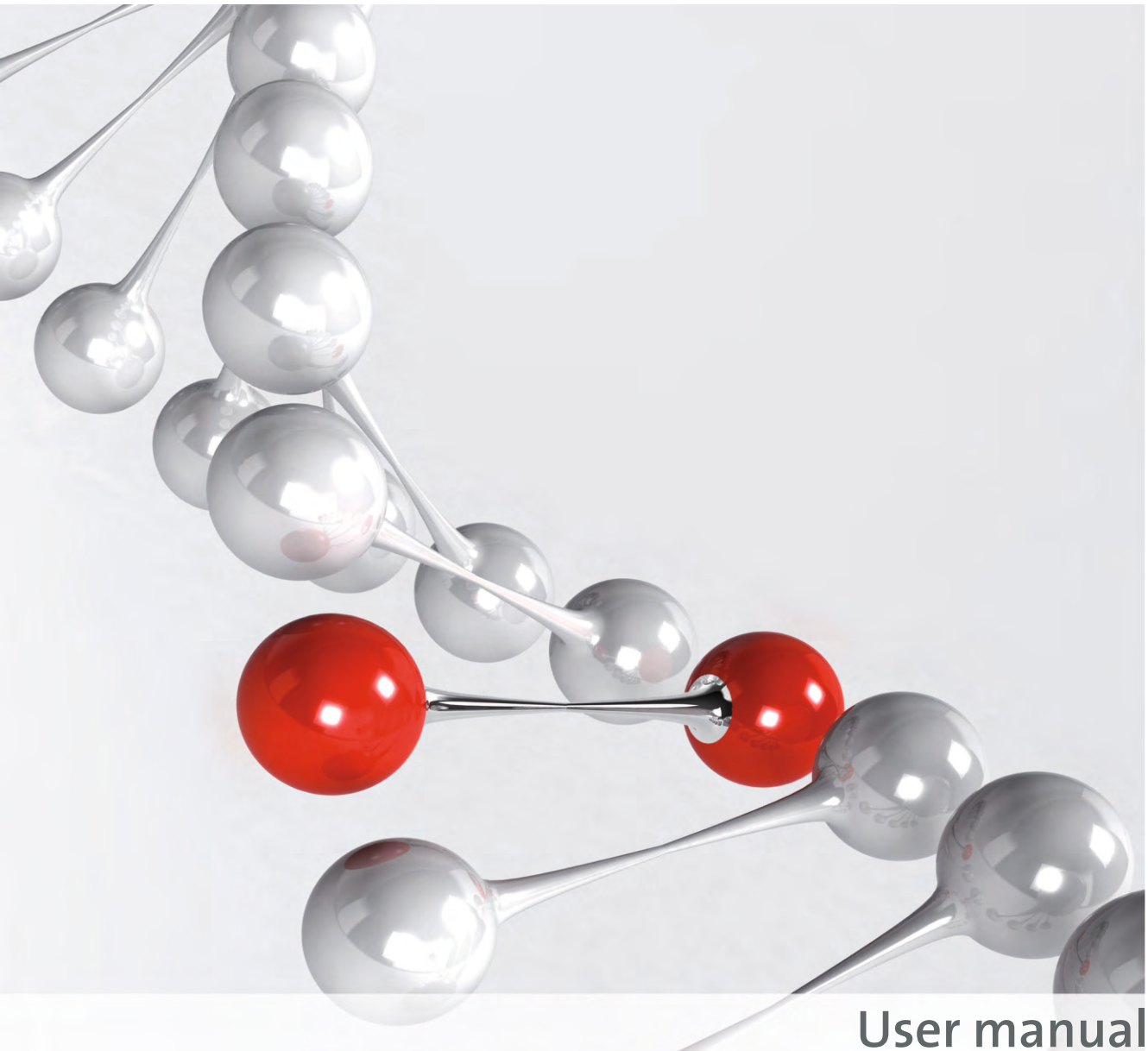


User manual
InviMag® Pathogen Kit/ KFmL
for use on KingFisher™ mL, Thermo Fisher Scientific

for automated purification of bacterial and viral DNA as well as viral RNA from human and animal serum, plasma samples, cerebrospinal fluid, cell-free body fluids as well as from swabs (rinse liquid) or tissue biopsies with magnetic beads

REF 2450110X00 STRATEC Molecular GmbH, D-13125 Berlin

Instruction InviMag® Pathogen Kit/ KFmL

The **InviMag® Pathogen Kit/ KFmL** is the ideal tool using a combination of RTP® technology and InviMag® technology for simultaneous isolation of high quality bacterial DNA as well as DNA and RNA from human and animal serum and plasma samples, cerebrospinal fluid, cell culture supernatants and other cell-free body fluids, fresh or frozen plasma or serum from blood treated with anticoagulants like EDTA or citrate, *but not with heparin*. The customer convenient RTP® technology simplifies the process handling, reduce the handling steps with infectious material and allow process monitoring.

Due to the high purity, the isolated DNA and RNA are ready-to-use for a broad panel of downstream applications or can be stored at -80°C for subsequent use.

The kit is neither suitable for isolation of bacterial DNA, viral DNA or viral RNA from whole blood or blood stains, nor for isolation of RNA or DNA from fungi or plants.

Content

Kit contents of InviMag® Pathogen Kit/ KFmL

	15 extractions	75 extractions	300 extractions
Catalogue No.	2445110100	2445110200	2445110400
Extraction Tube	15	75	6 x 50
Resuspension Buffer	15 ml	60 ml	150 ml
SNAP Solution	0.5 ml	2 x 1 ml	7 ml
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 8 ml)	empty bottle (final volume 40 ml)	empty bottle (final volume 140 ml)
Wash Buffer R1	10 ml (final volume 20 ml)	2 x 20 ml (final volume 2 x 40 ml)	2 x 80 ml (final volume 2 x 160 ml)
Wash Buffer R2	12 ml (final volume 60 ml)	30 ml (final volume 150 ml)	3 x 40 ml (final volume 3 x 200 ml)
Elution Buffer R	2 ml	15 ml	60 ml
KingFisher mL Tip Combs	3	15	60
KingFisher mL Tube Strips	15	5 x 15	300
Elution Tubes	15	5 x 15	6 x 50
Manual	1	1	1
Initial steps	Fill 8 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 10 ml of 96-100% ethanol to the bottle **Wash Buffer R1**, mix thoroughly and always keep the bottle firmly closed! Add 48 ml of 96-100% ethanol to the bottle **Wash Buffer R2**, mix thoroughly and always keep the bottle firmly closed!	Fill 40 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 20 ml of 96-100% ethanol to the bottle **Wash Buffer R1**, mix thoroughly and always keep the bottle firmly closed! Add 120 ml of 96-100% ethanol to the bottle **Wash Buffer R2**, mix thoroughly and always keep the bottle firmly closed!	Fill 140 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer R1**, mix thoroughly and always keep the bottle firmly closed! Add 160 ml of 96-100% ethanol to each bottle **Wash Buffer R2**, mix thoroughly and always keep the bottle firmly closed!

Kit contents of InviMag® Pathogen Kit/ KFmL / w/o plastic

	15 extractions	75 extractions	300 extractions
Catalogue No.	2445110150	2445110250	2445110450
Extraction Tube	15	75	6 x 50
Resuspension Buffer	15 ml	60 ml	150 ml
SNAP Solution	0.5 ml	2 x 1 ml	7 ml
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 8 ml)	empty bottle (final volume 40 ml)	empty bottle (final volume 140 ml)
Wash Buffer R1	10 ml (final volume 20 ml)	2 x 20 ml (final volume 2 x 40 ml)	2 x 80 ml (final volume 2 x 160 ml)
Wash Buffer R2	12 ml (final volume 60 ml)	30 ml (final volume 150 ml)	3 x 40 ml (final volume 3 x 200 ml)
Elution Buffer R	2 ml	15 ml	60 ml
Elution Tubes	15	5 x 15	6 x 50
Manual	1	1	1
Initial steps	Fill 8 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 10 ml of 96-100% ethanol to the bottle **Wash Buffer R1**, mix thoroughly and always keep the bottle firmly closed Add 48 ml of 96-100% ethanol to the bottle **Wash Buffer R2**, mix thoroughly and always keep the bottle firmly closed	Fill 40 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 20 ml of 96-100% ethanol to the bottle **Wash Buffer R1**, mix thoroughly and always keep the bottle firmly closed Add 120 ml of 96-100% ethanol to the bottle **Wash Buffer R2**, mix thoroughly and always keep the bottle firmly closed	Fill 140 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer R1**, mix thoroughly and always keep the bottle firmly closed Add 160 ml of 96-100% ethanol to each bottle **Wash Buffer R2**, mix thoroughly and always keep the bottle firmly closed
Plastic to be supplied by user (see order information)			
KingFisher mL Tip Combs	3	15	60
KingFisher mL Tube Strips	15	5 x 15	300

Symbols


Manufacturer


Lot number

Attention: *Do not combine components of different kits, unless the lot numbers are identical!*


Catalogue number


Expiry date


Consult operating instructions


Temperature limitation


Do not reuse


Humidity limitation

Storage

All buffers and kit contents of the **InviMag® Pathogen Kit/ KFmL** should be stored at room temperature and are stable for at least 12 months. Store the Kit especially the Extraction Tubes in a dry environment, the Extraction Tubes must be protected from humidity.

Room temperature (RT) is defined as range from 15-30°C.

Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by warming carefully (up to 30°C).

Wash Buffer charged with ethanol should be appropriately sealed and stored at room temperature.

Binding Solution (Isopropanol) should be appropriately sealed and stored at room temperature.

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **InviMag® Pathogen Kit/ KFmL** for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the product free of charge.
STRATEC Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.
In accordance with STRATEC Molecular's EN ISO 9001 and EN ISO 13485 certified Quality Management System the performance of all components of the **InviMag® Pathogen Kit/ KFmL** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.
If you have any questions or problems regarding any aspects of **InviMag® Pathogen Kit/ KFmL** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:
from Germany: +49-(0)30-9489-2901/ 2910
from abroad: +49-(0)30-9489-2907 or contact your local distributor.

Intended use

The **InviMag® Pathogen Kit/ KFmL** is the ideal tool for reliable and fast simultaneous isolation of high quality bacterial and viral DNA as well as viral RNA from fresh or frozen human or mammalian serum, plasma, cerebrospinal fluid, cell culture supernatants and other cell-free body fluids and swabs using the **RTP®-technology**, magnetic beads, and the KingFisher mL workstation.

The whole process is based on a patented technology, the **InviMag® technology**, for isolation of DNA and RNA by binding the nucleic acid onto magnetic particles without chaotropic buffer components.

For reproducible and high yields an appropriate sample storage and quick operation operation is essential. The purified viral DNA and/or RNA and bacterial DNA is ready-to-use for subsequent downstream applications.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA/RNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

Product use limitation

The kit is neither validated for the isolation of vertebrate nor parasite genomic DNA, total RNA, for the purification of pathogen nucleic acids from Heparin, EDTA or Citrate stabilized blood, for the purification of pathogen nucleic acids from Heparin stabilized serum or plasma.

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore neither a warranty no guarantee in this case will be given, implied or expressed.

The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the product with respect to specific applications.

STRATEC Molecular products may be used e.g. in clinical diagnostic laboratory systems under following conditions:

- If used in the US, based on the condition that the complete diagnostic system of the laboratory has been validated pursuant to CLIA' 88 regulations.
- For other countries based on the condition that the laboratory has been validated pursuant to equivalents according to the respective legal basis.

All products sold by STRATEC Molecular are subjected to extensive quality control procedures (according to EN ISO 9001 and EN ISO 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The product with its contents is unfit for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **InviMag® Pathogen Kit/ KFmL** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded accordingly to local safety regulations.

European Community risk and safety phrases for the components of the **InviMag® Pathogen Kit/ KFmL** to which they apply, are listed below as follows:

Extraction Tube

Warning

H302-H315-H319-H335-H411-P280-P305+P351+P338-EUH208

Wash Buffer R1

Warning

H302-H332-H412-P280-P305+P351+P338-EUH032

H302: Harmful if swallowed.
H315: Causes skin irritation.
H319: Causes serious eye irritation.
H332: Harmful if inhaled.
H335: May cause respiratory irritation.
H411: Toxic to aquatic life with long lasting effects.
H412: Harmful to aquatic life with long lasting effects.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
EUH208: Contains Proteinase, Tritirachium album-Serine. May produce an allergic reaction.
EUH032: Contact with acids liberates very toxic gas.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: 1 – 352 – 323 – 3500
inside of USA : 1 – 800 – 535 – 5053

Product characteristic of the InviMag® Pathogen Kit/ KFmL

Starting material	Yield	Time for preparation
up to 200 µl of fresh or frozen plasma, serum and cell free body fluids up to 200 µl cell culture supernatant up to 400 µl of rinse liquid from swabs 1 x 10^6 mammalian cells max. 10^9 bacteria	sensitive recovery realizing amplification with sensitive detection assays from starting material including minimum 100 copies per ml ***Note:*** The included Carrier-RNA will account for most of the eluted nucleic acids. Quantitative (RT)-PCR is recommended for determination of the RNA or DNA yield.	about 30 min after lysis

The **InviMag® Pathogen Kit/ KFmL** provides a fast and efficient tool for reliable simultaneous isolation of high quality viral DNA / RNA and bacterial DNA from a diverse range of clinical relevant samples, using a combination of RTP®-technology, magnetic beads and the KingFisher mL workstation.

The isolation process is based on a patented technology, the InviMag® technology, for isolation of viral RNA and DNA by binding the nucleic acids onto magnetic particles without chaotropic buffer components.

The kit uses the patented RTP® technology, whose special feature is the **Extraction Tube** containing already preformulated solid lysis reagent, lytic enzyme, Proteinase K, Carrier-RNA**.** The procedure is designed to avoid sample-to-sample cross-contaminations and allows safe handling of potentially infectious samples.

Using the **InviMag® Pathogen Kit/ KFmL** all types of samples are transferred into the **Extraction Tube** together with a specially designed **Resuspension Buffer** to adjust a final sample volume of 400 µl. The prefilled buffer and enzymes lyse the samples, stabilize the nucleic acids and enhance the viral and bacterial DNA and/ or RNA adsorption to the magnetic beads. Contaminants are removed by repeated washing steps and the purified nucleic acids are eluted in **Elution Buffer R**.

In addition to the rigorous lysis procedure, sample pretreating steps have been introduced, ideally for purification of genomic DNA of gram positive bacteria from different sources. The lysates are transferred to the subsequent automatic purification procedure based on magnetic beads. The DNA and RNA bind to magnetic particles, followed by washing steps and a final elution. The procedure requires only minimal interaction by the user, allowing safe handling of potentially infectious samples. The procedure is designed to avoid sample-to-sample cross-contamination. No phenol chloroform extraction or ß-Mercaptoethanol is required. All kit components (beside the SNAP Solution) can be stored at room temperature.

The advantage of the kit results from the simultaneous isolation of nucleic acids from DNA and RNA viruses as well as from bacteria. This allows the user to test the sample for all kind of nucleic acids from viruses and bacteria which are of interest after a preparation. High extraction efficiency and detection sensitivities are realized.

The purified high quality viral DNA and RNA is ready-to-use for subsequent downstream applications (see below) or can be stored at -20°C for subsequent use.

- RT-PCR*
- PCR*
- Real-time PCR for quantitative and qualitative virus detection

For the isolation of bacterial and viral nuceic acids from single samples using 200 µl starting material STRATEC Molecular offers the **RTP® Pathogen Kit**.
For the isolation of viral nucleic acids using magnetic particles in a high-throughput format, STRATEC Molecular offers the **InviMag® Pathogen Kits /KF96 /KFflex96** for use on a KingFisher 96 robot. For vacuum or centrifuge based isolation of viral NA in 96 well formats on different robotic stations STRATEC Molecular offer's the **Invisorb® DNA Virus HTS 96 Kit** and the **Invisorb® RNA Virus HTS 96 Kit** (see ordering information, page 28).

For further information please contact: +49 (0) 30 9489 2901, 2910 in Germany and from foreign countries: +49 (0) 30 9489 2907 or ask your local distributor.

Sampling and storage of starting material

Best results are obtained using freshly extracted samples. As long as the samples are not shock frosted with liquid nitrogen or are incubated with RNase inhibitors or denaturing reagents, the viral RNA is not secured. Therefore it is essential, that samples are immediately flash frozen subsequent to the harvesting by using liquid nitrogen and are stored at -80°C. Viral RNA contained in such deep frozen samples is stable for months. Viral RNA purification should be processed as soon as possible. Samples can also be stored in the dissolved Lysis Buffer in the Extraction Tube for 1 h at room temperature, overnight at 4°C, and for long-term storage at -80°C. Storage at deep frozen conditions is recommended.

Serum, plasma, urine, cerebrospinal fluid or other cell-free body fluids, as well as cell culture supernatants, swabs, and stool samples can be stored on ice for 1-2 hours, for short time (up to 24 h) samples may be stored at -20°C. For long-term storage, we recommend freezing samples at -20°C or – 80°C. Multiple thawing and freezing cycles before isolating the viral RNA should be avoided.

Cultivated bacteria:
Bacteria have to be pelleted after cultivation. Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C. Repeated freezing and thawing cycles of stored samples should be avoided because this may lead to degraded DNA.

Swabs, saliva:
The protocol works with fresh saliva, prepared swabs as well as with dried swabs. The protocol has not been validated for isolation of DNA from swabs which are stored in special storage buffers of other providers.

Serum and plasma (and other cell-free body fluids):
Following centrifugation, plasma or serum from blood treated with anticoagulants like EDTA or citrate, *but not with heparin*, can be stored at 2-8°C for up to 6 hours. For long-term storage, freezing at -20°C to -80°C in aliquots is recommended. Repeated freezing and thawing cycles should be avoided because denaturation and precipitation of proteins result in a decrease of the virus titer and thereby reduce the yield of the extracted nucleic acids. Occurring cryoprecipitates can be pelleted by briefly centrifuging (6.800 x *g* for 3 min). The cleared supernatant should be removed, without disturbing the pellet, and processed immediately. This step will not reduce viral titers.

Cell culture supernatants:
Best results are obtained with fresh material or material that has been immediately frozen and stored at -20°C or -80°C after separation of the cell culture supernatant. Repeated freezing and thawing cycles of stored samples will influence the sensitivity.

STRATEC Molecular will be released of its responsibilities if other sample materials than described in the Intended Use are processed or if the sample preparation protocols are changed or modified.

* The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

Principle and procedure

The **InviMag® Pathogen Kit/ KFmL** procedure comprises following steps:
- lysis of the bacterial and /or virus particles in the prefilled Extraction Tube
- binding the viral or bacterial nucleic acids to the magnetic beads
- washing of the magnetic beads and elimination of ethanol
- elution of the bacterial or viral nucleic acids

After lysis, the bacterial or viral nucleic acids bind to the magnetic beads whereas contaminations and enzyme inhibitors are efficiently removed during the following three wash steps and highly purified DNA/RNA is eluted in Elution Buffer R.
This manual contains 8 protocols.

Lysis
Samples are lysed at non-chaotropic conditions at different, elevated temperatures while continuously shaking. Lysis is performed in the presence of Lysozyme to break the cell wall of the bacteria while **Lysis Buffer** and **Proteinase K** digest proteins. All is provided prefilled in the **Extraction Tube.** Unlysed sample parts should be removed before the binding step. Due to the strong denaturing lyses conditions RNases and DNases are inactivated simultaneously.
The addition of **Carrier-RNA** (provided in the Extraction Tube) is necessary for the enhancement of viral DNA/ RNA recovery. Even very small number of viral DNA/ RNA molecules will also be purified. **Carrier-RNA** also stabilizes nucleic acids in samples with very small nucleic acid concentrations.

Binding of the bacterial and viral nucleic acids
After adding **Binding Solution** and **SNAP Solution** to the lysate, the nucleic acids are bound to the surface of the magnetic beads.

Removing residual contaminants
Contaminants are efficiently removed using **Wash Buffer R1** and **R2**, while the nucleic acids remain bound to the magnetic beads.

Elution
The nucleic acids are eluted from the beads using **Elution Buffer R**.
The eluted nucleic acids are ready-to-use in different subsequent tests.

Yield and quality of DNA and / or RNA

Different amplification systems vary in efficiency depending on the total amount of nucleic acids present in the reaction.
Eluates derived by this kit will contain Carrier-RNA, which will greatly exceed the amount of the isolated NA.
Yields of nucleic acids isolated from biological samples are usually low concentrated and therefore almost impossible to determine photometrically.*

* Keep in mind that the Carrier-RNA (5 µg per 200 µl sample) will account for most of the present NA.

The kit is suitable for downstream analysis, for examples qPCR, RT qPCR, LAMP, LCR. Diagnostic assays should be performed according to the manufacturer's instructions. The amount of purified viral nucleic acids in the **InviMag® Pathogen Kit/ KFmL** procedure from plasma etc. depends on the sample type, sample source, transport, storage and age.

Quantitative RT-PCR is recommended for determination of NA yield.

* In Gel Electrophoresis and in Capillary Electrophoresis, RNA extracted with the provided kit looks like degraded cause the kit contains Carrier-RNA, this is poly A RNA in fragments of 100 up to 1000 bases. The kit is not dedicated for applications using this kind of analysis.

Important notes

Important points before starting a protocol

Immediately upon receipt of the product, inspect the product and its components as well as the package for any apparent damages and correct quantities. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 7). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps are carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard contaminated gloves immediately.
- Do not combine components of different kits, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by trained personnel.

Preparing reagents and buffers

15 NA-extractions:
Fill 8 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 10 ml of 96-100% ethanol to the bottle **Wash Buffer R1**, mix thoroughly and always keep the bottle firmly closed! Add 48 ml of 96-100% ethanol to the bottle **Wash Buffer R2**, mix thoroughly and always keep the bottle firmly closed!
75 NA-extractions:
Fill 40 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 20 ml of 96-100% ethanol to the bottle **Wash Buffer R1**, mix thoroughly and keep the bottle always firmly closed Add 120 ml of 96-100% ethanol to the bottle **Wash Buffer R2**, mix thoroughly and always keep the bottle firmly closed!
300 NA-extractions:
Fill 140 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer R1**, mix thoroughly and always keep the bottle firmly closed! Add 160 ml of 96-100% ethanol to each bottle **Wash Buffer R2**, mix thoroughly and always keep the bottle firmly closed!

Important: *Some specific cell culture media may inhibit lysis efficiency or downstream reactions.*

Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Pipette and pipette tips
- Disposable gloves
- Reaction tubes (1.5 ml or 2.0 ml)
- Aqua dest.
- Vortexer
- 96–100% ethanol
- Isopropanol *

*The **InviMag® Pathogen Kit/ KFmL** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth**

*** Possible suppliers for Isopropanol:**

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no 59304-1L-F

Important indications

Preparing RNA

When preparing RNA, work quickly during the manual steps of the procedure.
The Lysis Buffer in the Extraction Tube simplifies RNA isolation by combining efficient lysis of the starting material and the inactivation of exogenous and endogenous RNases. Special care should be taken to avoid contaminations with RNases when handling Elution Buffer R.

Storing samples

Frozen serum or plasma samples must not be thawed more than once. Repeated freezing and thawing cycles lead to denaturation and precipitation of proteins, resulting in reduced titers and therefore reduced yields of nucleic acids.

Carrier-RNA

Carrier-RNA serves two purposes: It enhances the binding of acids to the beads, especially if there are only very few target molecules in the sample. Furthermore, the addition of large amounts of Carrier-RNA reduces the chance of nucleic acid degradation in the rare event that RNase or DNase molecules are not denaturated by the salts and detergents of the Lysis Buffer in the Extraction Tube.

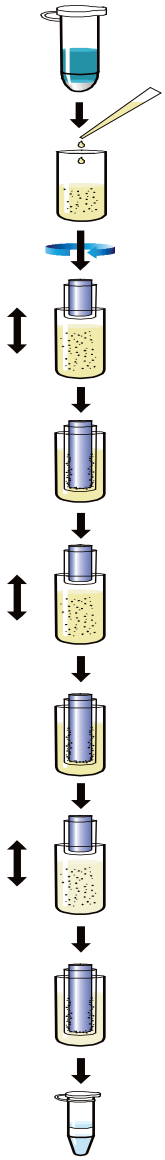
Internal Controls

Internal Controls (IC) from the PCR assay provider can be used as extraction controls if the fragments are longer than 100 bp.
The use of an internal extraction control is recommended when using the **InviMag® Pathogen Mini Kit/ KFmL** in combination with diagnostic amplification systems. The extraction control should be added after addition of the sample to the extraction tube. Never add the extraction control directly to the biological sample. The sample may include RNAses and DNAses which digest the extraction control.

Intercalating fluorescent dyes

Measurement of the DNA or RNA directly after isolation by intercalating fluorescent dyes may lead too low results. This is caused by the nature of the isolated DNA, due the denaturing step (95°C) during lysis DNA is partially single stranded. Therefore, fluorophores using double stranded DNA probes will not work correctly.

Scheme of the InviMag® Pathogen Kit/ KFmL



Before starting: Please read protocols very carefully and heat the thermomixers to 37°C, 65°C and 95°C.

Add sample to the Extraction Tube or resuspend sample pellets in 400 µl Resuspension Buffer,
for liquid samples adjust the total volume to 400 µl with Resuspension Buffer

Vortex for 10 s.
incubate for 10 min at 37°C in a thermomixer (optional)
incubate for 10 – 15 min. at 65°C in a thermomixer
incubate for 5 - 10 min. at 95°C in a thermomixer (optional)

During lysis, prefill all KingFisher tube strips with required buffers and appropriate volumes

Tube A: 400 µl **Binding Solution** and
20 µl **SNAP Solution**
Tube B: 800 µl **Wash Buffer R1**
Tube C: 800 µl **Wash Buffer R2**
Tube D: 800 µl **Wash Buffer R2**
Tube E: 100 µl **Elution Buffer R**

Add the lysed sample to Tube A

Start the instrument program InviMag_Pahogen_Kit

Viral or bacterial NA binding to magnetic particle

Magnetic separation

Washing of the particle fixed viral or bacterial NA

Magnetic separation

Elution of NA

Magnetic Separation

Pure NA

Protocol 1: Simultaneous isolation of total nucleic acids from cell free body fluids (serum, plasma, CSF)

Please read the instructions carefully and conduct the prepared procedure.

Sample Lysis

1. Transfer 200 µl of the sample into the **Extraction Tube** and add 200 µl of Resuspension Buffer. Close the cap and mix by vortexing for 10 s**.**
2. Place the **Extraction Tube** into a Thermomixer and incubate while continuously shaking for 10 min at 37°C.

   Attention please*: If sample contain only viral NA, incubation step 2 is not necessary.*
3. Place the **Extraction Tube** into another Thermomixer and incubate while continuously shaking for 10 -15 min at 65°C. During lysis, prefill all KF tube strips with the required buffers and appropriate volumes (see page 17).
4. Place the **Extraction Tube** into another Thermomixer and incubate while continuously shaking for 5 -10 min at 95°C.

   Attention please*: this step leads to higher sensitivity for some viruses e.g. HIV or HCV and is necessary for gram positive bacteria*
5. After lysis, transfer the lysed sample into the prefilled Tube A of the KingFisher tube strip (Binding Solution and **SNAP Solution** had been added before (see “Starting a run” page 17))
6. To start the program **InviMag_Pahogen_Kit**, see instructions on page 17.

Protocol 2: Simultaneous isolation of total nucleic acids from cell culture supernatant

Please read the instructions carefully and conduct the prepared procedure.

Sample Lysis

1. Transfer 200 µl of the cell free cell culture supernatant (cell culture media) into the **Extraction Tube** and add 200 µl Resuspension Buffer. Close the cap and mix by vortexing for 10 s.
2. Place the Extraction Tube into a Thermomixer and incubate while continuously shaking for 10 min at 37°C.

   Attention please*: If sample contain only viral NA, incubation step 2 is not necessary.*
3. Place the **Extraction Tube** into a Thermomixer and incubate while continuously shaking for 15 min. at 65°C. During lysis, prefill KF tube strips with the required buffers and appropriate volumes (see page 17).
4. Place the **Extraction Tube** into another Thermomixer and incubate while continuously shaking for 10 min at 95°C.

   Attention please*: this step leads to higher sensitivity for some viruses e.g. HIV or HCV and is necessary for gram positive bacteria*
5. After lysis, transfer the lysed sample into the prefilled Tube A of the KingFisher tube strip (Binding Solution, SNAP Solution had been added before (see “Starting a run” page 17))
6. To start the program **InviMag_Pahogen_Kit**, see instructions on page 17.

Protocol 3: Simultaneous isolation of total nucleic acids from swabs or 200 µl rinsed liquid

Please read the instructions carefully and conduct the prepared procedure

Sample Lysis

1a. **Use of the swab in the lysis**

Place the **swab** into the **Extraction Tube** and add 400 µl aqua dest.
Close the cap and mix by vortexing for 10 s**.**

Important Note: *To get maximum yields of viral nucleic acids it is essential to leave the swab during the complete lysis time in the reaction tube. Perform the lysis step with opened cap.It is also possible to cut off the shaft of the swab, and close the cap of the Extraction Tube. Removing the swab from the Extraction Tube ahead of time will lead to a dramatically reduced final yield! After lysis time carefully squeeze out the swab inside the wall of the tube and discard the swab.*

1b. **Use of rinsed liquid from swab**

a) the sample will also be used in the microbiology

Cut off the relevant part of the swab and transfer it into a RNase- and DNase-free 2 ml tube. Add 500 µl physiological saline solution and vortex intensely for 3 min. Transfer 400 µl of the rinsed liquid into the **Extraction Tube**. Close the cap and mix by vortexing for 10 s**.**

b) the sample will not be used in the microbiology

Cut off the relevant part of the swab and transfer it into a RNase- and DNase-free 2 ml tube. Add 500 µl RNase-free water to the swab and vortex intensely for 3 min.
Transfer 400 µl of the rinsed liquid into the **Extraction Tube**. Close the cap and mix by vortexing for 10 s**.**

1c. Use of transport media

Transfer 200 µl of stabilization media into the **Extraction Tube** and add 200 µl **Resuspension Buffer** Close the cap and mix by vortexing for 10 s**.**

2. Place the **Extraction Tube** into a Thermomixer and incubate while continuously shaking for 10min. at 37°C.

Attention please*: If sample contain only viral NA, incubation step 2 is not necessary.*

3. Place the **Extraction Tube** into a Thermomixer and incubate while continuously shaking for 15 min. at 65°C. During lysis, prefill all KF tube strips with the required buffers and appropriate volumes (see page 17).

4. Place the **Extraction Tube** into another Thermomixer and incubate while continuously shaking for 10 min at 95°C.

Attention please*:* *this step leads to higher sensitivity for some viruses e.g. HIV or HCV and is necessary for gram positive bacteria*

5. After lysis, transfer the lysed sample into the prefilled Tube A of the KingFisher tube strip (Binding Solution and **SNAP Solution** had been added before (see “Starting a run” page 17))

6. To start the program **InviMag_Pahogen_Kit**, see instructions on page 17.

Protocol 4: Isolation of DNA from bacteria pellets (maximum 10^9 bacteria cells)

Please read the instructions carefully and conduct the prepared procedure

Sample Lysis

1. Pellet the bacteria by centrifugation. Resuspend the bacterial pellet in 400 µl Resuspension Buffer R and transfer the resuspended sample into the **Extraction Tube** and vortex for 10 s.
2. Place the Extraction Tube into a Thermomixer and incubate while continuously shaking for 10 min at 37°C. Prefill all KF tube strips with the required buffers and appropriate volumes (see page 17).
3. Place the Extraction Tube into another Thermomixer and incubate while continuously shaking for 10-15 min at 65°C.
4. Place the Extraction Tube into another Thermomixer and incubate while continuously shaking for 5-10 min at 95°C.
5. After lysis, transfer the lysed sample into the prefilled Tube A of the KingFisher tube strip (**Binding Solution** and **SNAP Solution** had been added before (see "Starting a run" page 17))
6. To start the program **InviMag_Pahogen_Kit**, see instructions on page 17.

Protocol 5: Simultaneous isolation of total nucleic acids (DNA and RNA) from sputum

Please read the protocols carefully prior to the start of the preparation procedure!

Sample preparation

Transfer a defined aliquot e.g. 200 µl of the sputum sample into an RNase- and DNase-free tube and add the same volume e.g. 200 µl NAC Buffer (order number: 1033221100) or of saturated ACC solution to the sample (ratio sample to buffer must be 1:1). Incubate the mixture for 10 min at 95°C to reduce the viscosity.

Sample Lysis

1 Transfer 400 µl of the liquid sample into the **Extraction Tube** or 200 µl of a viscous sample and add 200 µl **Resuspension Buffer.**
Close the cap and mix by vortexing for 10 s**.**

2. Place the **Extraction Tube** into a Thermomixer and incubate while continuously shaking for 15 min.at 37°C.

Attention please*: If sample contain only viral NA, incubation step 2 is not necessary.*

3. Place the **Extraction Tube** into a Thermomixer and incubate while continuously shaking for 15 min. at 65°C. During lysis, prefill all KF tube strips with the required buffers and appropriate volumes (see page 17).

4. Place the **Extraction Tube** into another Thermomixer and incubate while continuously shaking for 10 min at 95°C.

Attention please*: this step leads to higher sensitivity for some viruses e.g. HIV or HCV and is necessary for gram positive bacteria*

5. After lysis, transfer the lysed sample into the prefilled Tube A of the KingFisher tube strip (**Binding Solution** and **SNAP Solution** had been added before (see "Starting a run" page 17))

6. To start the program **InviMag_Pahogen_Kit**, see instructions on page 17.

Protocol 6: Simultaneous isolation of total nucleic acids (DNA and RNA) from tracheal secretes or BAL

Please read the protocols carefully prior to the start of the preparation procedure!

Sample preparation

Non viscous samples:

Transfer a defined aliquot, e.g. 1 ml of trachialsecret or BAL, into a RNase- and DNase-free tube and centrifuge at 9.300 x *g* (1.0000 rpm) for 3 min. Discard the supernatant without disturbing the bacterial pellet: Resuspend the bacterial pellet in 400 µl distilled water or Resuspension Buffer.

Viscous sample

Transfer a defined aliquot, e.g. 1 ml of trachialsecret or BAL, into a RNase- and DNase-free tube and add the same volume, e.g. 1 ml NAC Buffer (order number: 1033221100) or of saturated ACC solution, to the sample (ratio sample to buffer must be 1:1)

Incubate the mixture for 10 min at 95°C to reduce the viscosity and centrifuge the sample at 9.300 x *g* (1.0000 rpm) for 3 min. Discard the supernatant without disturbing the bacterial pellet.

Resuspend the bacterial pellet in 400 µl distilled water or Resuspension Buffer

Sample Lysis

1. Transfer 400 µl of the liquid sample into the **Extraction Tube** or 200 µl of a viscous sample and add 200 µl aqua dest. Close the cap and mix by vortexing for 10 s**.**

2. Place the **Extraction Tube** into a Thermomixer and incubate while continuously shaking for 15 min.at 37°C.

   ***Attention please**: If sample contain only viral NA, this incubation step is not necessary*

3. Place the **Extraction Tube** into a Thermomixer and incubate while continuously shaking for 15 min. at 65°C. During lysis, prefill all KF tube strips with the required buffers and appropriate volumes (see page 17).

4. Place the **Extraction Tube** into another Thermomixer and incubate while continuously shaking for 10 min at 95°C.

   ***Attention please**: this step leads to higher sensitivity for some viruses e.g. HIV or HCV and is necessary for gram positive bacteria*

5. After lysis, transfer the lysed sample into the prefilled Tube A of the KingFisher tube strip (**Binding Solution** and **SNAP Solution** had been added before (see “Starting a run” page 17))

6. To start the program **InviMag_Pahogen_Kit**, see instructions on page 17.

Protocol 7: Simultaneous isolation of viral nucleic acids from stool samples

Please read the protocols carefully prior to the start of the preparation procedure!

Sample preparation

Transfer a defined aliquot, e.g. 100 µl of the stool sample, into a 2 ml tube and dilute the sample in a ratio 1:10 with RNase free water (Vortex the sample for 30 sec followed by a 1 min centrifugation step at 12.000 rpm (13.000 g).

Sample Lysis

1. Transfer 400 µl of the liquid sample into the **Extraction Tube**. Close the cap and mix by vortexing for 10 s**.**
2. Place the **Extraction Tube** into a Thermomixer and incubate while continuously shaking for 15 min. at 65°C. During lysis, prefill all KF tube strips with the required buffers and appropriate volumes (see page 17).
3. Place the **Extraction Tube** into another Thermomixer and incubate while continuously shaking for 10 min at 95°C. (These step leads to higher sensitivity for some viruses or some strains of viruses e.g. HIV or HCV,)
4. After lysis, transfer the lysed sample into the prefilled Tube A of the KingFisher tube strip (Binding Solution, SNAP Solution had been added before (see “Starting a run” page 17))
5. To start the program, see instructions on page 17.

Protocol 8: Isolation of bacterial DNA from stool samples

Please read the protocols carefully prior to the start of the preparation procedure!

Sample preparation

Transfer an defined aliquot e.g. 100 µl of the stool sample into a 2 ml tube and dilute the sample in a ratio 1:3 with RNase free water (300 µl). Vortex the sample for 30 sec followed by a 30 sec centrifugation step at 3.000 rpm (1.000 x *g*).

Sample Lysis

1. Transfer 200 µl of the supernatant of the stool suspension into the **Extraction Tube** and add 200 µl aqua dest. Close the cap and mix by vortexing for 10 s**.**
2. Place the **Extraction Tube** into a Thermomixer and incubate while continuously shaking for 15 min.at 37°C. During lysis, prefill all KF tube strips with the required buffers and appropriate volumes (see page 17).
3. Place the **Extraction Tube** into a Thermomixer and incubate while continuously shaking for 15 min. at 65°C.
4. Place the **Extraction Tube** into another Thermomixer and incubate while continuously shaking for 10 min at 95°C

   ***Attention please**:* *this step leads to higher sensitivity for some viruses e.g. HIV or HCV and is necessary for gram positive bacteria*

5. After lysis, transfer the lysed sample into the prefilled Tube A of the KingFisher tube strip (Binding Solution, SNAP Solution had been added before (see “Starting a run” page 17))
6. To start the program **InviMag_Pahogen_Kit**, see instructions on page 17.

Starting a run on the KF mL instrument

Please do not forget to place the KF mL Tip Combs in the guide rail of the Instrument

During lysis, prefill the tubes of the KingFisher tube strips with the required buffers and appropriate volumes.

KingFisher mL Tube Strip Setup

Note: ***Vortex SNAP Solution vigorously before use!***

Tube A**:** 400 µl **Binding Solution** and 20 µl **SNAP Solution** .

Tube B: 800 µl **Wash Buffer R1**

Tube C: 800 µl **Wash Buffer R2**

Tube D: 800 µl **Wash Buffer R2**

Tube E: 100 µl **Elution Buffer R**

After finishing the lysis step:

Tube A: transfer app. 450 µl of the **lysed sample**

1. Insert the prefilled KingFisher tube strips into the KingFisher instrument
2. Place the KingFisher tip combs onto the magnetic rack!

After these preliminary steps start the assay file "**InviMag_Pahogen_Kit**"

Important Notes:

After finishing the extraction protocol, the Tube E contains the extracted RNA / DNA. Store the RNA / DNA under adequate conditions.
We recommend transferring the extracted RNA / DNA into 1.5 ml reaction tubes and store the DNA / RNA at -20°C or -80°C (recommended for RNA).
If the nucleic acids contain carryover of magnetic particles, transfer the nucleic acids into a 1.5 ml reaction tube and centrifuge at maximum speed for 1 minute. Transfer the nucleic acid containing supernatant into a new tube.

The following steps will run automatically on the KingFisher™ mL

1. Binding of the DNA/RNA
Automatically sample mixing for 5 min.
SNAP-Bead separation.

2. Collect Beads
Automatically collection of Beads
Moving of the **SNAP-Beads** with bound nucleic acids into tube B.

3. First Washing
Automatically sample mixing for 1 min.
Automatically collection of Beads.
Moving of the **SNAP-Beads** with bound nucleic acids into tube C.

4. Second Washing
Automatically sample mixing for 1 min.
SNAP-Bead separation.
Moving of the SNAP-Beads with bound nucleic acids into tube D.

5. Third Washing and Drying
Automatically sample mixing for 1 min.
SNAP-Bead separation.
Drying the **SNAP-Beads** with bound nucleic acids outside of the tube for 8 min.
Moving of the **SNAP-Beads** into tube E

6. Elution of the DNA and / or RNA
Incubation of the SNAP-Beads in tube E for 10 min by mixing.
SNAP-Bead separation.
The **SNAP-Beads** will automatically be removed into tube D (disposal).

The extracted DNA/RNA can be transferred into 1.5 ml reaction tubes.

Optional: *Carryover of magnetic particles should be removed by centrifugation at max. speed for 1 min. Transfer the clear supernatant into a new 1.5 ml reaction tube.*

Note: *The eluate contains viral DNA and/or RNA. After extraction place the elution tube on ice. For a long-term freeze the nucleic acids at--20°C (DNA) or -80°C (recommended for RNA).*

For self-programming of the KingFisher™ mL system

Reagent info

A (Binding)

Name	Well volume [µl]	Total reagent volume [µl]	Type
Binding Solution	400	-	Reagent
SNAP Solution	20	-	Reagent
Lysed sample	400	-	Sample

B (Wash1)

Name	Well volume [µl]	Total reagent volume [µl]	Type
Wash Buffer R1	800	-	Reagent

C (Wash2)

Name	Well volume [µl]	Total reagent volume [µl]	Type
Wash Buffer R2	800	-	Reagent

D (Wash3)

Name	Well volume [µl]	Total reagent volume [µl]	Type
Wash Buffer R2	800	-	Reagent

E (Elution)

Name	Well volume [µl]	Total reagent volume [µl]	Type
Elution Buffer R	100	-	Reagent

Dispensed reagents

The protocol does not contain dispensed reagents

Steps data

Tip1		KingFisher ml tip comb	
	Binding	Pathogen_RTP	(A) - Binding
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium
	Mixing / pause:	Mixing time, speed	00:05:00, Medium
		Pause for manual handling	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	30
	CollectBeads1	Pathogen_RTP	(A) - Binding
		Collect count	5
		Collect time [s]	30
	CollectBeads2	Pathogen_RTP	(A) - Binding
		Collect count	5
		Collect time [s]	30
	Wash1	Pathogen_RTP	(B) - Wash1
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / pause:	Mixing time, speed	00:01:00, Fast
		Pause for manual handling	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	30
	CollectBeads3	Pathogen_RTP	(B) - Wash1
		Collect count	5
		Collect time [s]	30
	Wash2	Pathogen_RTP	(C) - Wash2
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / pause:	Mixing time, speed	00:01:00, Fast
		Pause for manual handling	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	30
	Wash3	Pathogen_RTP	(D) - Wash3
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / pause:	Mixing time, speed	00:01:00, Fast
		Pause for manual handling	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	30

	Drying	Pathogen_RTP	(D) - Wash3
		Dry time	00:08:00
		Tip position	Outside well / tube
	Elution	Pathogen_RTP	(E) - Elution
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium
	Mixing / pause:	Mixing time, speed	00:10:00, Slow
		Pause for manual handling	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	20
	Bead Removal	Pathogen_RTP	(D) - Wash3
		Release time, speed	00:00:30, Fast

Troubleshooting

Problem	Probable cause	Comments and suggestions
low amount of extracted DNA/RNA	insufficient lysis	increase lyses time, but prevent too long lyses times because this also decreases yield reduce amount of starting material
	incomplete elution	take higher volume of **Elution Buffer R**, be sure you pipet the **Elution Buffer R** with the right amount to the right position
	low amount of **SNAP Solution**	mix **SNAP Solution** thoroughly before pipetting to the KingFisher tube
low concentration of extracted DNA/RNA	too much Elution Buffer	elute the DNA with lower volume of Elution Buffer R
	incorrect storage of starting material	ensure that the storage of starting material was correct avoid thawing of the material
degraded or sheared DNA/RNA	incorrect storage of starting material	ensure that the storage of starting material was correct avoid thawing of the material
	old material	ensure that the starting material is fresh or stored under appropriate conditions (for long time storage at –20°C)! avoid thawing and freezing of the material old material often contains degraded DNA
DNA/RNA does not perform well in downstream-applications (e.g. real-time PCR or PCR)	salt carryover during elution	check **Wash Buffers** for salt precipitates. If there are any precipitates visible, solve them by carefully warming up to 30°C ensure that the **Wash Buffers** are equilibrated at room temperature
low A_{260}:A_{280} ratio from UV measurement, eluted DNA is brown colored	small part of the magnetic particles are left in the elution	centrifuge at full speed for 1 min and transfer supernatant into a new tube

Appendix

KingFisher™ BindIt Software 3.2 or higher versions

BindIt software 3.2 or higher versions were and may be used to create assay files for the KFmL, KF96/KFflex96 or KF-Duo instruments. The provided assay file(s) can either be transferred onto the corresponding workstation(s) or be started directly from within the BindIt software after assay import. Please keep in mind, that assay(s) run from within the BindIt software are not stored in the workstation memory.

Important: *Be advised that BindIt SW 3.2 or higher versions use a new unique file extension. Therefore, it is not possible to import assay files created with BindIt 3.2 or higher versions into older BindIt software versions! Please ask your local Thermo Scientific distributor for a software update.*

***Note**:* *When creating assay files for usage with KingFisher instruments in combination with Microtiter Deep Well plates (e.g. Thermo Electron), it is essential to use the KingFisher software 3.2 or higher versions for assay development because this software version includes the correct adjustments for the microtiter plate. It is highly recommended to use Thermo Microtiter Deep Well plates with KF96 / KFflex96 / KF-Duo workstations to ensure the best purification result.*

Minimum system requirements for BindIt Software 3.2 or higher versions

PC requirements	
Supported operating systems	MS Windows XP Pro with SP3, Windows Vista SP2, Windows 7
Disk space	500 MB free disk space
Processor	Intel Pentium ≥ 1 GHz
Memory	1 GB RAM
Serial ports available	1 (for KFmL connection)
USB ports available	1 (for KF96 / KFflex96 / KFDuo connection)
Pointing device	Mouse or equivalent is required
CD-ROM drive	1
Monitor / color settings	XVGA monitor with at least 1024x768 resolution and at least a 16-bit color environment

If the actual Windows Service Packs are not installed on the corresponding lab computer, they can be downloaded from the Microsoft web pages: http://www.microsoft.com/

General notes on handling DNA

Nature of DNA
The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long-template PCR.

Storage of DNA
A working stock of DNA can be stored at 2-8˚C for several weeks. For long-term storage DNA should be stored at -20˚C. However, storage at -20°C may cause shearing, particularly if the DNA is exposed to repeated freeze-thawing cycles.

Note that the solution in which the nucleic acid is eluted in will affect it's stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

Dissolving and pipetting DNA
Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

DNA yield
The amount of purified DNA depends on sample source, transport conditions, storage, and age of the sample.

General notes on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases has to be reduced as much as possible. Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification is carried out.

All glassware should be treated before use to ensure that it is RNase-free. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240°C for four or more hours before use. Autoclaving alone will not completely inactivate many RNases. Oven baking will both inactivate RNases and ensure that no other nucleic acids (such as Plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1% DEPC (diethyl pyrocarbonate). The glassware must stand 12 hours at 37°C and then be autoclaved or heated to 100°C for 15 min to remove residual DEPC.

- **Electrophoresis tanks** should be cleaned with detergent solution (e.g. 0.5% SDS), thoroughly rinsed with RNase-free water followed by ethanol and allowed to dry.
- **Non-disposable plastic ware** should be treated before use to ensure that it is RNase-free. Plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. Alternatively, chloroform-resistant plastic ware can be rinsed with chloroform to inactivate RNases.
- All **buffers** must be prepared with RNase-free ddH_2O.
- When **working with chemicals**, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Change gloves frequently and keep tubes closed.
- All centrifugation steps are carried out at room temperature.
- To avoid **cross-contaminations,** cavity seams shouldn't be moistened with fluid.
- Reduce the preparation time as much as possible.
- **Use only sterile, disposable polypropylene tubes** throughout the procedure. (The tubes are generally RNase-free.)
- Keep isolated RNA on ice.
- Do not merge kit components from other kits unless the lot numbers are identical.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.

This kit should only be used by trained personnel.

Storage of RNA

Purified viral RNA can be stored at -80°C and is stable for several years at this condition.

Ordering information

Product	Package Size	Catalogue No.
InviMag® Pathogen Kit / KFmL	75 preparations	2445110200
InviMag® Pathogen Kit / KFmL	300 preparations	2445110400
InviMag® Pathogen Kit / KFmL / w/o plastic	75 preparations	2445110250
InviMag® Pathogen Kit / KFmL / w/o plastic	300 preparations	2445110450
Related Products		
InviMag® Pathogen Kit /KF96 KFflex96	1 x 96 preparations	7444050100
InviMag® Pathogen Kit /KF96 KFflex96	5 x 96 preparations	7444050200
RTP® Pathogen Kit	50 preparations	1040500200
RTP® Pathogen Kit	250 preparations	1040500300
RTP® DNA/ RNA Virus Mini Kit	50 preparations	1040100200
RTP® DNA/ RNA Virus Mini Kit	250 preparations	1040100300
RTP® Bacteria DNA Mini Kit	50 preparations	1033200200
RTP® Bacteria DNA Mini Kit	250 preparations	1033200300
Invisorb® Virus RNA HTS 96 Kit /X	4 x 96 preparations	7143310300
Invisorb® Virus RNA HTS 96 Kit /X	24 x 96 preparations	7143310400

Ordering information (KingFisher™ mL and consumables)

Cat.no	Description
5400050	KingFisher mL, Magnetic Particle Processor, 100-240 V, 50/60 Hz
97002111	KingFisher mL tip comb, 800 pcs
97002121	KingFisher mL tube, 900 pcs (20x45 pcs)
97002131	KingFisher mL Combi 60 (tubes and tip combs for 60 samples)
97002141	KingFisher mL Combi 240 (tubes and tip combs for 240 samples)

Possible suppliers for Isopropanol

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no 59304-1L-F

stratec molecular

STRATEC Molecular GmbH
Robert-Rössle-Str. 10
13125 Berlin, Germany

Phone: +49 30 94 89 29 01
Fax: +49 30 94 89 29 09
E-mail: info.berlin@stratec.com

www.stratec.com

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