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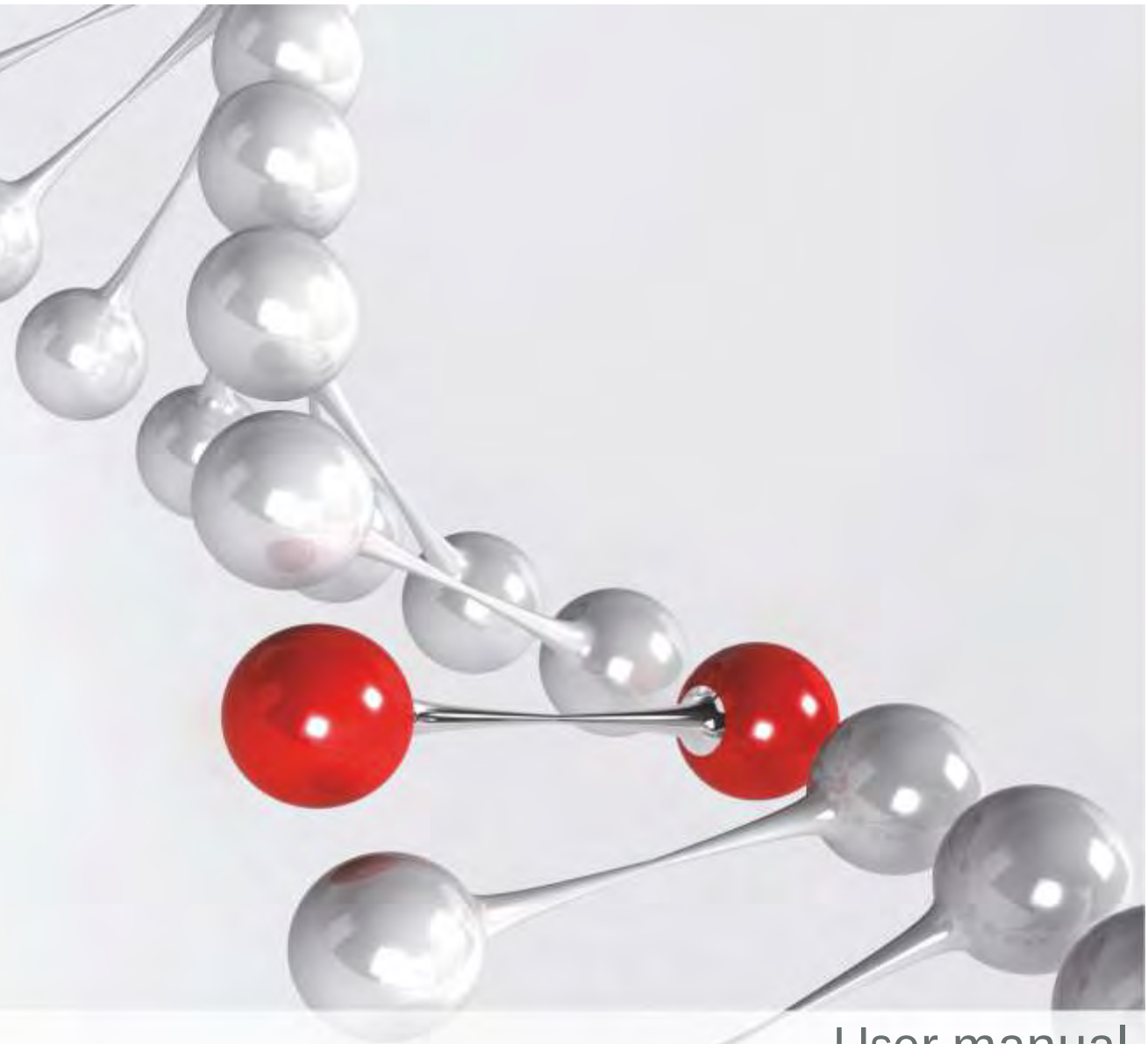


User manual
InviMag® Pathogen Kit/ KF96

for use on KingFisher™ 96 and KingFisher™ Flex, Thermo Fisher Scientific

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

REF 7444050X0 STRATEC Molecular GmbH, D-13125 Berlin

Instruction for the InviMag® Pathogen Kit/ KF96

The **InviMag® Pathogen Kit/ KF96** combines the advantages of the innovative RTP® technology with easy handling of magnetic particles for a very efficient and reliable isolation of nucleic acids with a high purity.
The **InviMag® Pathogen Kit/ KF96** is the optimal tool for simultaneous isolation of high-quality genomic bacterial DNA and viral DNA/RNA from human and animal serum, plasma and other cell-free body fluids - like urine - as well as from swabs (rinse liquid), sputum, tracheal secretes, BAL, tissue biopsies or stool samples in a convenient 96 well format using the KF96 or KFflex96 workstation from Thermo Fisher Scientific.
Fresh or frozen plasma and serum derived from blood treated with anticoagulants like EDTA or Citrate, but ***not*** heparin, can be used.
The interplay of the DNA/RNA extraction and purification chemistry provided by the **InviMag® Pathogen Kit/ KF96** with the KingFisher™ instrument was intensely tested and validated.

The customer convenient RTP® technology, using prefilled Extraction Bottles, simplifies the process handling, reduces manual handling steps with infectious material and allows process monitoring. The nucleic acid (NA) binding particles (magnetic beads) are characterized by a high surface area, uniform size distribution, good suspension stability and therefore are highly suitable for high-throughput processing.

The kit is neither validated for the isolation of genomic DNA from stool samples and parasites nor for the purification of total RNA from clinical samples.

For research use only!

Table of content

Kit contents of InviMag® Pathogen Kit/ KF96

	1x 96 preps	5x 96 preps
Catalogue No.	7444050100	7444050200
Extraction Bottle (50)	2	10
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 60 ml)	empty bottle (final volume 250 ml)
Resuspension Buffer R	30 ml	120 ml
SNAP Solution	2 x 1.1 ml	10.5 ml
Wash Buffer R1	80 ml (final volume 160 ml)	3 x 80 ml (final volume 3 x 160 ml)l
Wash Buffer R2	40 ml (final volume 200 ml)	4 x 40 ml (final volume 4 x 200 ml)
Elution Buffer R	15 ml	60 ml
KF96 Tip Comb for DW magnets	1	5
2.0 ml Deep Well Plate	4	4 x 5
200 µl Elution Plate	2	5 x 2
Sealing Foils	2	10
Manual	1	1
Receiver tubes	2 x 50 pieces	10 x 50 pieces
Initial steps	Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer R1**, mix thoroughly and keep the bottle firmly closed! Add 160 ml of 96-100% ethanol to the bottle **Wash Buffer R2**, mix thoroughly and keep the bottle firmly closed! Resuspend each **Extraction Bottle (50)** in 10 ml Resuspension Buffer R Fill 60 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 keep the bottle firmly closed! Add 160 ml of 96–100% ethanol to each bottle **Wash Buffer R2**, mix thoroughly and keep the bottle firmly closed! Resuspend each **Extraction Bottle (50)** in 10 ml of Resuspension Buffer R Fill 250 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle

Kit contents of InviMag® Pathogen Kit/ KF96 w/o plastic

	1x 96 preps	5x 96 preps
Catalogue No.	7444050100	7444050200
Extraction Bottle (50)	2	10
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 60 ml)	empty bottle (final volume 250 ml)
Resuspension Buffer R	30 ml	120 ml
SNAP Solution	2 x 1.1 ml	10.5 ml
Wash Buffer R1	80 ml (final volume 160 ml)	3 x 80 ml (final volume 3 x 160 ml)
Wash Buffer R2	40 ml (final volume 200 ml)	4 x 40 ml (final volume 4 x 200 ml)
Elution Buffer R	15 ml	60 ml
Sealing Foils	2	10
Manual	1	1
Receiver tubes	2 x 50 pieces	10 x 50 pieces
Initial steps	Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer R1**, mix thoroughly and keep the bottle firmly closed! Add 160 ml of 96-100% ethanol to the bottle **Wash Buffer R2**, mix thoroughly and keep the bottle firmly closed! Resuspend each **Extraction Bottle (50)** in 10 ml Resuspension Buffer R Fill 60 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 keep the bottle firmly closed! Add 160 ml of 96–100% ethanol to each bottle **Wash Buffer R2**, mix thoroughly and keep the bottle firmly closed! Resuspend each **Extraction Bottle (50)** in 10 ml of Resuspension Buffer R Fill 250 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle
Plastic to be supplied by user (see order information)		
2.0 ml Deep Well Plate	4 pieces	5 x 4 pieces
KF 96 Tip Comb for DW magnets	1 piece	5 pieces
200 µl Elution Plate	2 pieces	5 x 2 pieces

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/ KF96** should be stored at room temperature and are stable for at least 12 months. Store the Kit especially the Extraction Bottles in a dry environment, the Extraction Bottles 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 Buffers 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/ KF96** 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/ KF96** 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/ KF96** 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:
Germany: +49-(0)30-9489-2901/ 2910
Abroad: +49-(0)30-9489-2903/ 2907 or contact your local distributor.

Intended use

The **InviMag® Pathogen Kit/ KF96** is designed for a simultaneous rapid and economical preparation of DNA and/or RNA from viruses and genomic DNA from bacteria derived from fresh or frozen human or mammalian serum, plasma, and other cell-free body fluids - like urine - as well as from swabs (rinse liquid), sputum, tracheal secretes, BAL, tissue biopsies or stool samples using the **RTP® technology** and a KingFisher workstation.

The whole process is based on a patented technology, the **InviMag® technology**, which relies on binding of the nucleic acids onto magnetic particles in absence of chaotropic buffer components. The procedure only requires minimal user interaction, allowing safe handling of potentially infectious samples.

The isolation protocols and buffers are optimized to provide high yields and purities. However, for reproducible yields an appropriate sample storage and quick handling is essential. The purified viral DNA and/or RNA as well as bacterial DNA are ready-to-use for subsequent analysis.

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 tested nor validated for the isolation of genomic DNA from vertebrates or parasites, total RNA, for purification of pathogen nucleic acids from heparin, EDTA or citrate stabilized blood nor for the 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 nor 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 subject 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 plastic are for laboratory use only. They must be stored in the laboratory and must not be used for other purposes than intended.

The product with its contents is not suitable 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.molecular.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/ KF96** 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 and handled as infectious and discarded accordingly to local safety regulations.

European Community risk and safety phrases for the components of the **InviMag® Pathogen Kit/ KF96** to which they apply are listed below as follows:

Extraction Bottle

Warning

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

Wash Buffer R1 (Prämix)

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/ KF96

Starting material	Yield	Time
up to 200 µl liquid sample up to 1 x 10^9 bacteria cells up to 10 mg tissue sample	depends on the sample (storage and source) ***Note:*** The added Carrier RNA will account for most of the eluted nucleic acid(s). Quantitative (RT)-PCR is recommended for determination of the viral RNA or DNA yield.	70 minutes (includes lysis)

The **InviMag® Pathogen Kit/ KF96** provides a fast and efficient tool for a reliable, simultaneous isolation of high quality viral DNA/RNA and/or genomic bacterial DNA from a diverse range of starting materials. The procedures described within this manual are suitable for plasma or serum (that either contains Citrate or EDTA, but **not** heparin), other cell-free body fluids like urine, tissue samples, swabs, sputum, tracheal secrete, BAL and stool samples. Samples can be used either fresh or frozen. However, samples should not be thawed and frozen more than once because this may lead to degraded nucleic acids.

The amount of purified DNA and/or RNA with the **InviMag® Pathogen Kit/ KF96** procedures depends on the sample type, sample source, transport, storage, age and pathogen titer.

The kit uses the patented RTP® technology, whose special feature is the **Extraction Bottle (50)** that contains a preformulated solid lysis reagent, Lysozyme, Proteinase K and Carrier RNA. The procedure is designed to avoid sample-to-sample cross-contaminations and allows safe handling of potentially infectious samples with only minimal user interaction.

In addition to the rigorous lysis procedure, sample pretreating steps for purification of genomic DNA from Gram-positive bacteria have been introduced.
The advantage of the kit results from the simultaneous isolation of nucleic acids from DNA and RNA viruses as well as DNA from bacterial species. This allows sample testing in regard to all kinds of nucleic acids from viruses and bacteria which are of interest after a preparation. High extraction efficiency and detection sensitivities are realized. No phenol chloroform extraction or ß-Mercaptoethanol is required. The working time required for the whole procedure is reduced to a minimum.
Yield and quality of the isolated nucleic acids are suitable for any detection system. The tests should be performed accordingly to the manufacturer's specifications. The isolated viral DNA/RNA and bacterial DNA are ready-to-use in a broad panel of downstream applications or can alternatively be stored at -80°C for subsequent use.

- (RT)-PCR*
- Real-time PCR (quantitative (RT)-PCR, like TaqMan und LightCycler technology)
- cDNA synthesis
- Microarray application
- RFLP-Analysis

*) The PCR method is covered by U.S. Patents 4,683,195 and 4,683,202 owned by Hoffmann-LaRoche Inc. The purchase of the RTP® Pathogen Kit /KF96 KFflex96 cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-LaRoche Inc.

Important: *Keep in mind, that kits which are simultaneously purifying DNA and RNA in general show less precise results than the corresponding kits designed for isolation of one specific type of nucleic acid.*

To purify highly chromosomal bacterial DNA in a 96 well format, STRATEC Molecular offers the **Invisorb® Universal Bacterial HTS 96 Kit** for use in a centrifuge and on common automated laboratory workstations. STRATEC Molecular also offers systems for the purification of viral DNA and/or RNA from up to 200 µl sample volume – the **RTP® Virus DNA/RNA Mini Kit**. In combination with magnetic beads the **InviMag® Virus DNA/RNA Mini Kit /KFmL** and **InviMag® Virus DNA/RNA Mini Kit /KF96** are available.

For the separate isolation of viral DNA or RNA from serum and plasma in single tube or 96 well format – the **Invisorb® Spin Virus RNA Mini Kit** or **Invisorb® Spin Virus DNA Mini Kit** are available. The **Invisorb® Virus RNA HTS 96 Kit/ X** is designed for use on X-tractor Gene™ (Corbett Robotics) and the **InviMag® Virus RNA Kit KF96** for use on a KingFisher™ instrument.

If you are interested in using the kit on a different laboratory workstation or if you require additional technical support or further information, please do not hesitate to contact us: phone +49 (0) 30-9489-2901/ -2907/ -2910/ -2903 or ask your local distributor.

Internal control (IC) / Extraction control

Internal controls (IC) from the PCR assay provider can be used as extraction controls. In this case they have to be added to the lysate of the clinical sample following the instructions after finalization of the lysis step. If it only should be used as inhibition control and has to be added to the final eluted RNA/DNA.

Attention: Don´t add the internal controls directly to the sample!

Sampling and storage of starting material

Cultivated bacteria or bacterial suspension(s):
Bacteria have to be pelleted after cultivation. Best results are obtained with fresh material or material that has been immediately shock frosted 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 nucleic acids.

Biopsy material/ tissue:
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 nucleic acids. Use of poor quality starting material also leads to reduced length and influences the yield of purified nucleic acids. The amount of purified DNA from up to 10 mg tissue sample depends on the nature of starting material. The thawing process can be prolonged, e.g. in the Extraction Bottle.

Urine:
The bacteria must be pelleted while the supernatant is discarded (urea contaminations can inhibit PCR reactions). Best results are obtained with freshly pelleted material or bacterial pellets that have been immediately frozen and stored at -20°C or -80°C. Repeated freezing and thawing cycles of frozen samples should be avoided because this may lead to degraded nucleic acids. The amount of purified DNA from 15-50 ml urine depends on the bacterial titer.

Swabs, saliva:
The protocol works with fresh saliva, prepared swabs as well as with dried swabs. The protocol is not validated for the isolation of DNA from swabs using storage buffers from other providers.

Best results are obtained using freshly extracted samples. As long as the samples are not shock frosted in liquid nitrogen or 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 harvesting by using liquid nitrogen and storage at -80°C. Viral RNA in deep frozen samples is stable for months. Viral RNA purification should be processed as soon as possible. Samples can be stored alternatively in dissolved Lysis Buffer in the Extraction Bottle for 1 h at room temperature, overnight at 4°C, and for long-term storage at -80°C.

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*** heparin, can be stored at 2-8°C for up to 6 hours. For long-term storage, freezing at -20°C or -80°C in aliquots is recommended. Repeated freezing and thawing cycles must be avoided due to denaturation and precipitation of proteins resulting in a decrease of the virus titer and thereby reducing the yield of the extracted viral RNA. Occurring cryoprecipitates can be pelleted by briefly centrifugation (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.

Stool samples:
Best results are obtained with fresh material. Stool samples contain DNases and RNases which quickly realize DNA and RNA digestion and degradation. The sample may be stored frozen at – 80°C.

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.

Principle

The **InviMag® Pathogen Kit/ KF96** procedure comprises the following steps:
1. Lysis at different temperatures
2. Adjustment of the binding conditions
3. Binding of the nucleic acids to magnetic particles
4. Washing of the bead bound nucleic acids and evaporation of ethanol
5. Elution of the nucleic acids

This manual contains 4 protocols.

Procedure

Bacteria must be cultivated at special conditions. An aliquot of the bacteria suspension is used to achieve a bacterial pellet by centrifugation at high speed for 5 min. The supernatant is discarded.

Lysis
Samples are lysed at non-chaotropic conditions at different elevated temperatures while continuously shaking. Lysis is performed in the presence of **Lysozyme** and **Lysis Buffer** to break bacterial and viral cell walls whereas **Proteinase K** is used for digestion of proteins. All reagents are provided prefilled in the **Extraction Bottle (50).** Due to the strong denaturing lyses conditions RNases and DNases are inactivated simultaneously.

The addition of **Carrier RNA** (a component of Extraction Bottle (50)) is necessary for the enhancement and stabilization of viral DNA/RNA recovery. Due to this, it is even possible to purify very small amounts of viral DNA/RNA molecules.

Binding of nucleic acids
After adding **Binding Solution** to adjust optimal binding conditions, the released nucleic acids are bound by the simultaneously added magnetic beads.

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

Elution
High quality viral DNA/RNA and genomic bacterial DNA are finally eluted from the beads using **Elution Buffer R**. The elution volume is 100 µl. An elution in a larger volume (up to 200 µl) is possible but will lead to a dilution of the DNA/RNA yield. An elution volume of less than 100 µl is not recommended. The eluted DNA/RNA is ready-to-use in different subsequent applications.

Note: *If the elution volume is modified, this has to be manually adjusted in the provided assay file too!*

Protocol validation

The pathogen extraction protocol was intensively tested on a KingFisher™ Flex 96 instrument with the provided reagents and consumables. Typical results for the extraction of bacterial DNA, viral DNA and RNA are shown below. Actual results can vary, depending upon sample age, quality, type, and the species used.

Samples

For testing the isolation efficiency of bacterial DNA, frozen cell pellets from the gram-positive bacterium *Bacillus subtilis* were used in dilution from $1x10^9$ to $1x10^4$. The bacteria were grown in an over-night culture and the derived cell pellets from 1 ml of this culture were stored at -20°C until further use. In all experiments a fresh pellet was used from the -20°C stock. The detection was done by an in-house *Bacillus subtilis* real-time PCR based detection assay performed on a Step One Plus Cycler (Applied Biosystems). For the extraction process 200 µl of the corresponding dilution was used, respectively.
For testing of viral DNA and RNA, 200 µl plasma was spiked with 2 µl hCMV or 2 µl Influenza stock solution, respectively. The detection was done by an in-house real-time PCR for hCMV and Influenza, respectively. All real-time PCR's were performed on a Corbett Rotor-Gene 3000 or Step One Plus Cycler (Applied Biosystems).

Bacterial Detection (Bacillus subtilis)

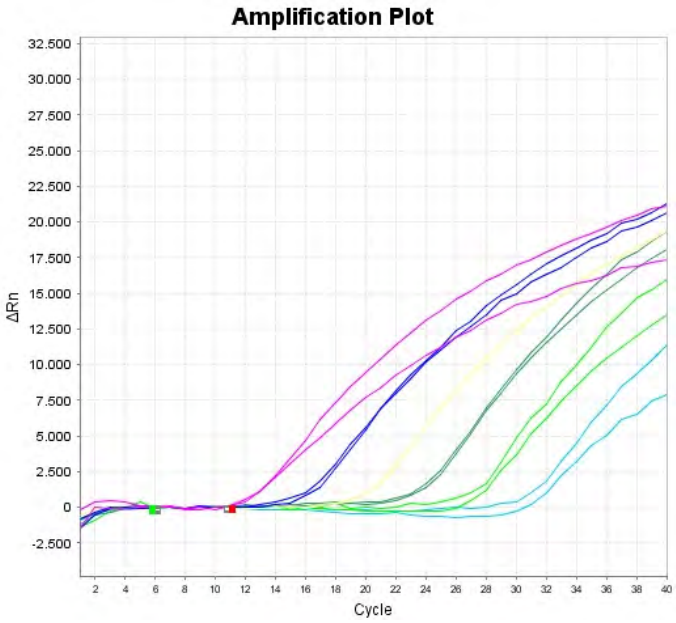


Fig. 1: Real-time PCR results from a representative RT-PCR run with *Bacillus subtilis* samples performed in dilutions (10^9 purple, 10^8 blue, 10^7 yellow, 10^6 dark green, 10^5 light green, 10^4 cyan).

Sample	Cт	Cт SD
B.s. 10^9	13,909	0,041
B.s. 10^9	13,851	0,041
B.s. 10^8	17,403	0,225
B.s. 10^8	17,085	0,225
B.s. 10^7	21,256	0,096
B.s. 10^7	21,120	0,096
B.s. 10^6	24,635	0,205
B.s. 10^6	24,345	0,205
B.s. 10^5	28,606	0,368
B.s. 10^5	28,085	0,368
B.s. 10^4	32,868	0,559
B.s. 10^4	32,077	0,559

Tab. 1: The table shows the average Ct values and the corresponding standard deviation of *Bacillus subt*. spiked samples derived by real-time PCR.

DNA Virus Detection (*hCMV*)

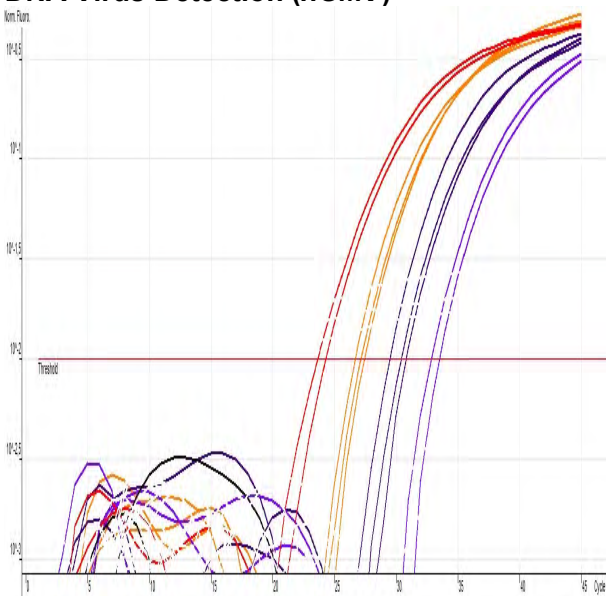


Fig. 2: Real-time PCR results from a representative RT-PCR run with *hCMV* spiked samples performed in dilutions (undiluted (yellow), 10^{-1} (dark blue), 10^{-2} (purple), PTC (red), NTC (black).

Color	Name	Type	Ct
	hCMV, undiluted	Sample	27,46
	hCMV, undiluted	Sample	27,18
	hCMV, undiluted	Sample	26,72
	hCMV, 10^{-1}	Sample	30,82
	hCMV, 10^{-1}	Sample	30,43
	hCMV, 10^{-1}	Sample	29,50
	hCMV, 10^{-2}	Sample	32,87
	hCMV, 10^{-2}	Sample	
	hCMV, 10^{-2}	Sample	33,52
	PTC	Positive Control	24,32
	PTC	Positive Control	23,68
	NTC	NTC	
	NTC	NTC	

Tab. 2: The table shows the estimated Ct values of *human Cytomegalovirus* spiked samples performed in dilutions and derived by real-time PCR.

RNA Virus Detection (*Influenza*)

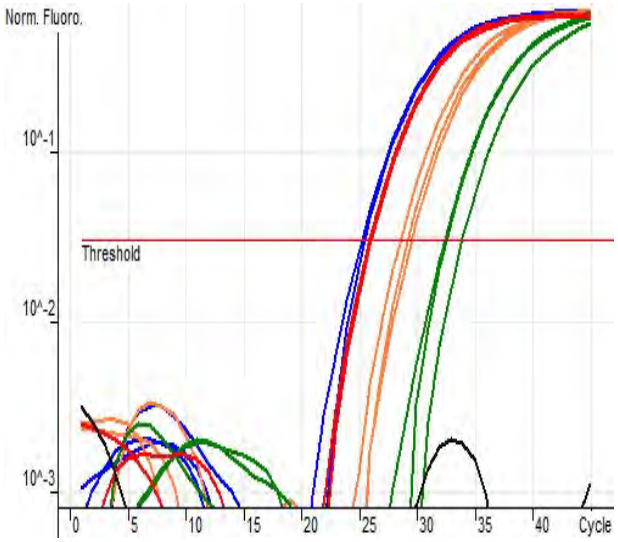


Fig. 3: Real-time PCR results from a representative RT-PCR run with *Influenza* spiked samples performed in dilutions (undiluted (dark blue), 10^{-1} (orange), 10^{-2} (green), PTC (red), NTC (black).

Color	Name	Type	Ct
	Infl., undiluted	Sample	25,56
	Infl., undiluted	Sample	25,30
	Infl., undiluted	Sample	26,01
	Infl., 10^{-1}	Sample	28,64
	Infl., 10^{-1}	Sample	29,63
	Infl., 10^{-1}	Sample	29,27
	Infl., 10^{-2}	Sample	32,46
	Infl., 10^{-2}	Sample	33,98
	Infl., $10e^{-2}$	Sample	32,61
	PTC	Positive Control	25,96
	PTC	Positive Control	26,15
	NTC	NTC	

Tab. 3: The table shows the estimated Ct values of *Influenza* spiked samples performed in dilutions and derived by real-time PCR.

Important indications

Carrier RNA

Carrier RNA, included in the Extraction Bottle (50), serves two purposes: It enhances the binding of viral acids to the magnetic beads, especially if there are only very few target molecules present in the sample and reduces the chance of viral nucleic acid degradation in case that RNases or DNases present in the sample.

Yield and quality of pathogen DNA/RNA

Different amplification systems vary in efficiency, depending on the total amount of nucleic acids present in the sample. Eluates derived by this kit will contain Carrier-RNA, which will greatly exceed the amount of the isolated NA.
Yields of viral 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 RNA. .

The kit is suitable for downstream analysis with NAT techniques like qPCR, RT-qPCR, LAMP and LCR. Diagnostic assays should be performed accordingly to the manufacturer's instructions.

Quantitative PCR is recommended for determination of viral yields.

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

Use of internal control

If an internal extraction control should be used, please add the control during the pause step after lysis when the Binding Solution and beads have to be added. Adding the control directly to the sample before lysis may lead to a degradation of the internal control if not all nucleic acid related proteinases are inactivated by the lysis solution. The instrument will pause after lysis to allow adjustment of the binding conditions ("Add Isopropanol and beads" step).

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's, please consult the appropriate material safety data sheets (MSDS) (see our webpage: **www.molecular.stratec.com**).

1. Microcentrifuge ≥ 9.300 x *g* (≥ 10.000 rpm), optional
2. Distilled water
3. Ethanol (96-100%)
4. 1.5 ml reaction tubes, optional
5. Measuring cylinder (250 ml)
6. Disposable gloves
7. Pipet with tips (we highly recommend to use filter tips only)
8. 15 or 50 ml reaction tubes, optional
9. Isopropanol*

*) The **InviMag® Pathogen Kit/ KF96** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth.**

Possible suppliers for Isopropanol:

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

Fa. Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

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

Important notes

Immediately upon receipt of the product, inspect the product and its components as well as the package for any apparent damages, correct quantities and quality. 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, because their use may lead to poor kit performance.

1. Always change pipet tips between liquid transfers. To avoid cross-contaminations, we recommend the use of aerosol-barrier pipet tips.
2. All centrifugation steps are carried out at room temperature.
3. When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
4. Discard contaminated gloves immediately.
5. Do not combine components of different kits unless the lot numbers are identical.
6. Avoid microbial contamination of the kit reagents.
7. 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 personal.

Preparing buffers

1 x 96 extractions:
Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer R1** and mix thoroughly Add 160 ml of 96-100% ethanol to the bottle **Wash Buffer R2** and mix thoroughly. Resuspend each **Extraction Bottle (50)** in 10 ml of DNase/RNase free water Fill 60 ml **Isopropanol** (molecular biologic grade) into the empty bottle
5 x 96 extractions:
Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer R1** and mix thoroughly. Add 160 ml of 96-100% ethanol to each bottle **Wash Buffer R2** and mix thoroughly. Resuspend each **Extraction Bottle (50)** in 10 ml of DNase/RNase free water Fill 250 ml **Isopropanol** (molecular biologic grade) into the empty bottle

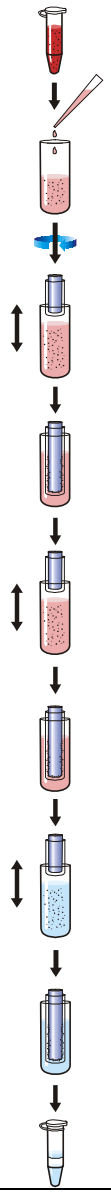
Scheme of the InviMag® Pathogen Kit/ KF96

Please read protocols prior the start of the preparation carefully.

Transfer 200 µl solution from the **Extraction Bottle (50)** and 200 µl **sample** into a cavity of a 2 ml Deep Well Plate (refers as "Lysis Plate"). Continue with the respective lysis protocol (see pages 16).

Prefill all plates with required buffers and appropriate volumes.

Tip Plate:	Insert the KF96 Tip Comb for DW magnets on a Tip Plate*
Lysis Plate:	Add 200 µl **Lysis solution** from Extraction Bottle (50) and 200 µl **sample**
Washing Plate_1:	Add 800 µl **Wash Buffer R1** to a 2.0 ml Deep Well Plate
Washing Plate_2:	Add 800 µl **Wash Buffer R2** to a 2.0 ml Deep Well Plate
Washing Plate_3:	Add 800 µl **Wash Buffer R2** to a 2.0 ml Deep Well Plate
Elution Plate:	Add 100 µl **Elution Buffer R** to the Elution Plate (same size as Tip Plate)



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

The following steps are performed on the KingFisher instrument:

Lysis of the sample

After lysis, during the pause step, please add **500 µl Binding Solution** and **20 µl SNAP Solution**

Important: *If an internal extraction control should be used, please add it to the reaction mixture at this step*

Nucleic acids bind to magnetic particles

Washing of the particle fixed nucleic acids

Magnetic separation

Elution of nucleic acids

Magnetic Separation

Pure nucleic acids

* Elution Plates and Tip Plates are identically. Use one provided Elution Plate as a Tip Plate.

Protocol 1: Simultaneous isolation of total nucleic acids (DNA and RNA) from cell-free body fluids

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

Important Note: *The protocol has been optimized for a sample volume of 200 µl. For smaller samples volumes than 200 µl please adjust to a total volume of 200 µl with ddH_2O or PBS.*

Transfer 200 µl prepared Lysis Solution Mixture from the **Extraction Bottle (50)** into a cavity of a 2 ml Deep Well Plate (refers as "Lysis Plate") and add 200 µl sample.

Prefill the remaining plates with required buffers and appropriate volumes and continue with the loading of the system (see "Starting a Run", page 19).

Protocol 2: Simultaneous isolation of total nucleic acids (DNA and RNA) from swab material

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

Transfer 200 µl prepared Lysis Solution Mixture from the **Extraction Bottle (50)** and 200 µl distilled water into a cavity of a 2 ml deep well plate (refers as "Lysis Plate"). Insert the swab into the cavity of the Lysis Plate and mix by stirring with the swab. Incubate for 5-10 min at RT and stir occasionally. After incubation, remove the swab by squeezing out the swab inside the cavity to remove residual liquid and then discard the swab.

Prefill all remaining plates with the required buffers and appropriate volumes and continue with the loading of the system (see "Starting a Run", page 19).

Protocol 3: Simultaneous isolation of total nucleic acids (DNA and RNA) from tissue biopsies

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

Transfer 1-10 mg from the tissue biopsy sample into a cavity of a 2ml Deep Well Plate (refers as "Lysis Plate") and add 200 µl distilled water and 200 µl Lysis Solution Mixture from the **Extraction Bottle (50).**

Prefill the remaining plates with required buffers and appropriate volumes and continue with the loading of the system (see "Starting a Run", page 19).

Protocol 4: Isolation of DNA from bacteria pellets ($1x10^9$ bacteria cells)

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

Take an aliquot of the bacteria culture and centrifuge the sample at 9.300 x *g* (10.000 rpm) for 3 min. Discard the supernatant without disturbing the bacterial pellet.

Resuspend the bacterial pellet in 200 µl distilled water or PBS and transfer the sample into a cavity of a 2 ml Deep Well Plate (refers as "Lysis Plate"). Add 200 µl Lysis Solution Mixture from the **Extraction Bottle (50)**.

Prefill the remaining plates with required buffers and appropriate volumes and continue with the loading of the system (see "Starting a Run", page 19).

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!

Transfer an defined aliquot e.g. 150 µl of the sputum sample into an RNAse and DNAse free tube and add the same volume e.g. 150 µl NAC Buffer (order number: 1033221100) or 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 transfer 200 µl of the sample into the cavity of a 2 ml Deep Well Plate (refers as “Lysis Plate”). Add 200 µl Lysis Solution Mixture from the **Extraction Bottle (50)**.

Prefill the remaining plates with required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 19).

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!

Non viscous samples:

Transfer an defined aliquot e.g. 1 ml of tracheal secrete or BAL into an RNAse and DNAse free tube and centrifuge the sample at 9.300 x *g* (10.000 rpm) for 3 min. Discard the supernatant without disturbing the bacterial pellet. Resuspend the bacterial pellet in 200 µl distilled water or PBS and transfer the sample into a cavity of a 2 ml Deep Well Plate (refers as “Lysis Plate”). Add 200 µl Lysis Solution Mixture from the **Extraction Bottle (50)**.
Prefill the remaining plates with required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 19).

Viscous sample:

Transfer an defined aliquot e.g. 1 ml of tracheal secrete or BAL into an 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* (10.000 rpm) for 3 min. Discard the supernatant without disturbing the bacterial pellet.

Resuspend the bacterial pellet in 200 µl distilled water or PBS and transfer the sample into a cavity of a 2 ml Deep Well Plate (refers as “Lysis Plate”). Add 200 µl Lysis Solution Mixture from the **Extraction Bottle (50)**.

Prefill the remaining plates with required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 19).

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

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

Transfer an defined aliquot e.g. 100 µl of the stool sample into a 2 ml tube and adjust the sample with RNAse free water to 1 ml**. (Attention**: if you want to use more sample please adjust with RNAse free water in a ratio: 1:10) Vortex the sample for 30 sec followed by a 1 min centrifugation step at 12.000 rpm (13.000 g).

Transfer 200 µl of the virus containing supernatant into the cavity of a 2 ml Deep Well Plate (refers as “Lysis Plate”). Add 200 µl Lysis Solution Mixture from the **Extraction Bottle (50)**.

Prefill the remaining plates with required buffers and appropriate volumes and continue with the loading of the system (see “Starting a Run”, page 19).

Protocol 8: Isolation of bacterial DNA from stool samples

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

Transfer an defined aliquot e.g. 100 µl of the stool sample into a 2 ml tube and dilute the sample with RNAse free water to 300 µl **(Attention**: if you want to use more sample please adjust with RNAse free water in a ratio: 1:3). Vortex the sample for 30 sec followed by a 30 sec centrifugation step at 3.000 rpm (1.000 g).

Transfer 200 µl of the bacteria containing supernatant into the cavity of a 2 ml Deep Well Plate (refers as "Lysis Plate"). Add 200 µl Lysis Solution Mixture from the **Extraction Bottle (50)**.

Prefill the remaining plates with required buffers and appropriate volumes and continue with the loading of the system (see "Starting a Run", page 19).

Starting a Run

I. Preliminary Steps to process the sample onto the KingFisher System

Important: *For working with the KF96 / KFflex96 instrument, please carefully read the manufacturer´s manual before using the system for the first time!*

1. Switch on the KF96 / KFflex96 instrument

a. **Tip Plate**: Place the KF96 Tip Comb for DW magnets on an Elution Plate (Tip Plate).

Note*: Use one provided Elution Plate as Tip Plate. These are identical.*

2. Prefill the all Deep Well Plates with the required buffers and appropriate volumes

Important: *Mix the bottle with the **SNAP Solution** by vigorously vortexing before usage!*

Lysis Plate: Add 200 µl **Sample** and 200 µl **Lysis Solution** (from Extraction Bottle (50))
Washing plate_1: Add 800 µl **Wash Buffer RI** into the cavities of a Deep Well Plate
Washing plate_2: Add 800 µl **Wash Buffer RII** into the cavities of a Deep Well Plate
Washing plate_3: Add 800 µl **Wash Buffer RII** into the cavities of a Deep Well Plate
Elution Plate: Add 100 µl **Elution Buffer R** into the cavities of a Elution Plate

3. Choose the running file"**InviMag_Pathogen_KF96"** or **"InviMag_Pathogen_KFflex96"** on the display of the instrument – depends on the used instrument - and press the "START" button.
4. Insert the prefilled plates onto the right position of the KingFisher surface by following the specification printed on display. Confirm every step by pressing the "START" button.
5. If all prefilled plates are added to the system press the "START" button again to initialize the assay file.
6. After the lysis steps, a pause will occur and 500 µl **Binding Solution** and 20 µl **SNAP Solution** have to be added to each used cavity of the Lysis Pate. If extraction controls are used, please add them at this step.
7. Reinsert the plate into the instrument (watch out for correct plate orientation) and continue the run by pressing the "START" button. The instrument will now continue with the purification process without any further user interaction.

II. The following steps run automatically on the KingFisher System

1. Lysis

Lysis steps at 45°C for 10 min, 70°C for 10 min and 95°C for 5 min are performed. After lysis, the instrument will be paused and the user has to add 500 µl **Binding Solution** and 20 µl **SNAP Solution** (magnetic beads). Internal extraction controls should be added during the pause step too.

2. Binding of the DNA

Automatically sample mixing for 10 min. SNAP separation. Transportation of the SNAP bound nucleic acids into Washing Plate 1.

2. First Washing Step

Automatically sample washing for 1 min. SNAP separation. Transportation of the SNAP bound nucleic acids into the Washing Plate 2.

3. Second Washing Step

Automatically sample washing for 1 min. SNAP separation. Transportation of the SNAP bound nucleic acids into the Washing Plate 3.

4. Third Washing and Drying Step

Automatically sample washing for 1 min. SNAP separation. Drying the SNAP bound nucleic acids outside the plate for 3.5 min. Transportation of the SNAP into the Elution Plate.

5. Elution of the DNA

Incubation of the SNAP bound nucleic acids into the Elution Plate for 10 min by mixing at elevated temperature. SNAP separation. The SNAP without the bound nucleic acids are afterwards automatically discarded into the wells of Washing Plate_3 (disposal).

If necessary, the extracted DNA can be transferred manually into 1.5 ml Receiver Tubes (provided).

Important Notes: *After finishing the extraction protocol, the Elution Plate contains the extracted nucleic acids. Store the nucleic acids at adequate conditions. We recommend transferring the extracted nucleic acids into 1.5 ml Receiver Tubes (provided) and store them at -20° C or –80°C.*

If the extracted DNA contains carryover of magnetic particles, transfer the eluate into a 1.5 ml reaction tube, centrifuge at maximum speed for 1 min and then transfer the DNA-containing supernatant into the provided Receiver Tubes.

The eluted DNA is ready-to-use in different downstream applications.

For self-programming of the KF96 and KFflex96 instrument

Reagent info

Tip Plate		KingFisher 96 KF plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Lysis Plate		**Microtiter DW 96 plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Sample	200	-	Sample
Extraction Solution	200	-	Reagent
Washing Plate 1		**Microtiter DW 96 plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer RI	700 → Flex 800 →KF96	-	Reagent
Washing Plate 2		**Microtiter DW 96 plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer RII	800	-	Reagent
Washing Plate 3		**Microtiter DW 96 plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer RII	800	-	Reagent
Elution Plate		**KingFisher 96 KF plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Elution Buffer R	100	-	Reagent

Dispensed reagents

Lysis Plate		Microtiter DW 96 plate	
Name	**Step**	**Well volume [µl]**	**Total reagent volume [µl]**
Isopropanol	Adjust Binding	500	-
SNAP Solution	Adjust Binding	20	-

Steps data

Tip1		96 DW tip comb	
	Pick-Up	Tip Plate	
	Lysis Step 1	Lysis Plate	
	Beginning of step	Precollect	No
		Release beads	No
	Mixing / heating:	Mixing time, speed	00:10:00, Medium
		Heating temperature [°C]	45
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
	Lysis Step 2	Lysis Plate	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:10:00, Medium
		Heating temperature [°C]	70
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
	Lysis Step 3	Lysis Plate	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating temperature [°C]	95
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
	Adjust Binding	Lysis Plate	
		Message	Add Binding Solution and
		Dispensing volume [µl]	520
	Reagent(s)	Name	Binding Solution
		Volume [µl]	500
		Name	SNAP Solution
		Volume [µl]	20
	Binding Step	Lysis Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:10:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	10

	Wash Step 1	Washing Plate 1	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	5
	Wash Step 2	Washing Plate 2	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	5
	Wash Step 3	Washing Plate 3	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	5
	Drying Step	Washing Plate 3	
		Dry time	00:03:30
		Tip position	Outside well / tube
	Elution Step	Elution Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium
	Mixing / heating:	Mixing time, speed	00:10:00, Slow
		Heating temperature [°C]	65
		Preheat	Yes
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	15
	Bead Removal Step	Washing Plate 3	
		Release time, speed	00:00:30, Fast
	Leave	Tip Plate	

Troubleshooting

Problem	Probable cause	Comments and suggestions
low amount of extracted DNA	insufficient lysis	increase lysis time, but prevent too long lysis time because this also decreases yield reduce amount of starting material
	incomplete elution	increase the volume of **Elution Buffer R**, ensure that the **Elution Buffer R** is transferred into the right position; change the modified volume in the provided assay file too
	low amount of SNAP Solution	mix **SNAP Solution** vigorously before use
low concentration of extracted DNA	too much Elution Buffer	elute the DNA with in a lower volume of **Elution Buffer R**. Change the modified volume in the run file too.
	incorrect storage of starting material	ensure that the storage of starting material was correct. avoid repeated thawing and freezing cycles of the sample material
	incorrect Wash Buffers	make sure, that the correct amount of ethanol is added to the **Wash Buffers** and stored correctly
degraded DNA	incorrect storage of starting material	ensure that the storage of starting material was correct ensure that the starting material is stored at appropriate conditions (–20°C) avoid multiple thawing and freezing cycles of the material
DNA does not perform well in downstream-applications (e.g. real-time PCR or PCR)	ethanol carryover during elution	increase drying time for removal of ethanol in the assay file
	salt carry-over during elution	check the **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
eluted DNA is brownish colored	small part of the magnetic particles are left in the elution	centrifuge at full speed for 1 min and transfer supernatant to a new tube

Problem	Probable cause	Comments and suggestions
low amount of extracted RNA	insufficient lysis	increase lyses time, but prevent too long lyses time because this decrease the yield reduce amount of starting material
	incomplete elution	use a higher volume of **Elution Buffer R**. make sure you pipet the **Elution Buffer R** with the correct volume to the right position. change the modified volume in the provided assay file too
	low amount of SNAP Solution	mix **SNAP Solution** thoroughly before pipetting to the Deep Well Plate
low concentration of extracted RNA	too much Elution Buffer R	elute the RNA with lower volume of Elution Buffer R;. change the modified volume in the run file too
	incorrect storage of starting material	ensure that the storage of starting material was correctly avoid repeated thawing of the material
	incorrect Wash Buffers	make sure that the correct amount of ethanol is added to the **Wash Buffers**
degraded RNA	incorrect storage of starting material	ensure that the storage of starting material was correctly avoid thawing of the material
	old material	ensure that the starting material is fresh or stored at appropriate conditions (-80 C) avoid multiple thawing and freezing of the material
RNA does not perform well in downstream-applications (e.g. real-time RT-PCR or RT-PCR)	ethanol carryover during elution	increase drying time for removing of ethanol in the assay file
	salt carryover during elution	check the **Wash Buffers** for salt precipitates. If there are any precipitates, solve them by carefully warming up to 30°C ensure that the **Wash Buffers** are equilibrated at room temperature
eluted RNA is brownish colored	small parts 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 it will work well in various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long-template PCR, and construction of cosmid libraries.

Handling fresh and stored material before the extraction of DNA
For the isolation of genomic DNA from cells or tissues, use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at -70°C. This procedure minimizes degradation of crude DNA by limiting the activity of endogenous nucleases.

Storage of DNA
Store DNA at 2 - 8°C. Storage of genomic DNA at -20°C may cause shearing, particularly if the DNA is exposed to repeated freezing and thawing cycles.

Drying, dissolving and pipetting DNA
Avoid overdrying genomic DNA after ethanol precipitation. It is better to air-dry DNA than to use a vacuum, although vacuum drying can be used with caution.

To help dissolve the DNA, carefully invert the tubes several times after adding buffer and tap the tube gently on the side. Alternatively incubate the DNA in buffer overnight at 2 - 8°C. Minimize vortexing of genomic DNA because this can cause shearing.

Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings can cause 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. Regular pipette tips pose no problem for plasmid and other small DNA.

Quantification
Quantification of DNA and RNA from this assay must be done by means of qPCR or Reverse Transcriptase qPCR. All other methods will be disturbed by the included Carrier Nucleic Acids as well as DNA or RNA which is co-purified.

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 have to be reduced to a minimum. 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 must be RNase free. Therefore, the glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240°C for four or more hours before use. Autoclaving only 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 react 12 hours at 37°C and should 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, rinsed with ethanol and allowed to air-dry.
- **Non-disposable plastic ware** should be treated before use to ensure that it is RNase free. Plastics should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase free water. You can also take chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.
- All solutions must be prepared with RNase free water.
- Change gloves frequently and keep tubes closed.
- When handling RNA, reduce the preparation time as much as possible.
- Only use sterile disposable polypropylene tubes throughout the procedure.
- Always keep RNA samples on ice.

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.

Quantification
Quantification of DNA and RNA from this assay must be done by means of qPCR or Reverse Transcriptase qPCR. All other methods will be disturbed by the included Carrier Nucleic Acids as well as DNA or RNA which is co-purified.

Ordering information

Product	Package Size	Catalogue No.
InviMag® Pathogen Kit /KF96	1 x 96 preparations	7444050100
InviMag® Pathogen Kit /KF96	5 x 96 preparations	7444050200

Related Products	Package Size	Catalogue No.
RTP® Pathogen Kit	250 preparations	1040500300
RTP® DNA/ RNA Virus Mini Kit	250 preparations	1040100300
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
InviMag® Virus RNA Kit/ KF 96	5 x 96 preparations	7443300200
InviMag® Virus DNA/ RNA Mini Kit/ KFmL	75 preparations	2441150200
InviMag® Bacteria DNA Mini Kit/ KFmL	75 preparations	2433150200
Invisorb® Universal Bacteria HTS 96 Kit	24 x 96 preparations	7033310400

Possible suppliers for Isopropanol:

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

Fa. Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

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

Possible suppliers for centrifuges:

Eppendorf AG
22331 Hamburg, Germany
Phone.: +49 (0) 40 53801 0
Fax: +49 (0) 40 53801 556
E-Mail: eppendorf@eppendorf.com
Internet: www.eppendorf.com

SIGMA Laborzentrifugen GmbH
37507 Osterode am Harz, Germany
Phone.: +49-5522-5007-0
Fax: +49-5522-5007-12
E-Mail: info@sigma-zentrifugen.de
Internet: www.sigma-zentrifugen.de

KingFisher™ 96 and consumables

KingFisher 96, Magnetic Particle Processor,100-240V,50/60Hz	5400500
(including one magnetic head)	
KingFisher 96 Head for Deep Well plate	24073430
KingFisher 96 tip comb for PCR magnets, 8 x 10 pcs / box	97002514
KingFisher 96 tip comb for KF magnets, 10 x 10 pcs / box	97002524
KingFisher 96 tip comb for DW magnets 10 x 10 pcs / box	97002534
KingFisher 96 KF plate (200ul) 48 plates / box	97002540
Microtiter deep well 96 plate, 50 plates/box	95040450

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