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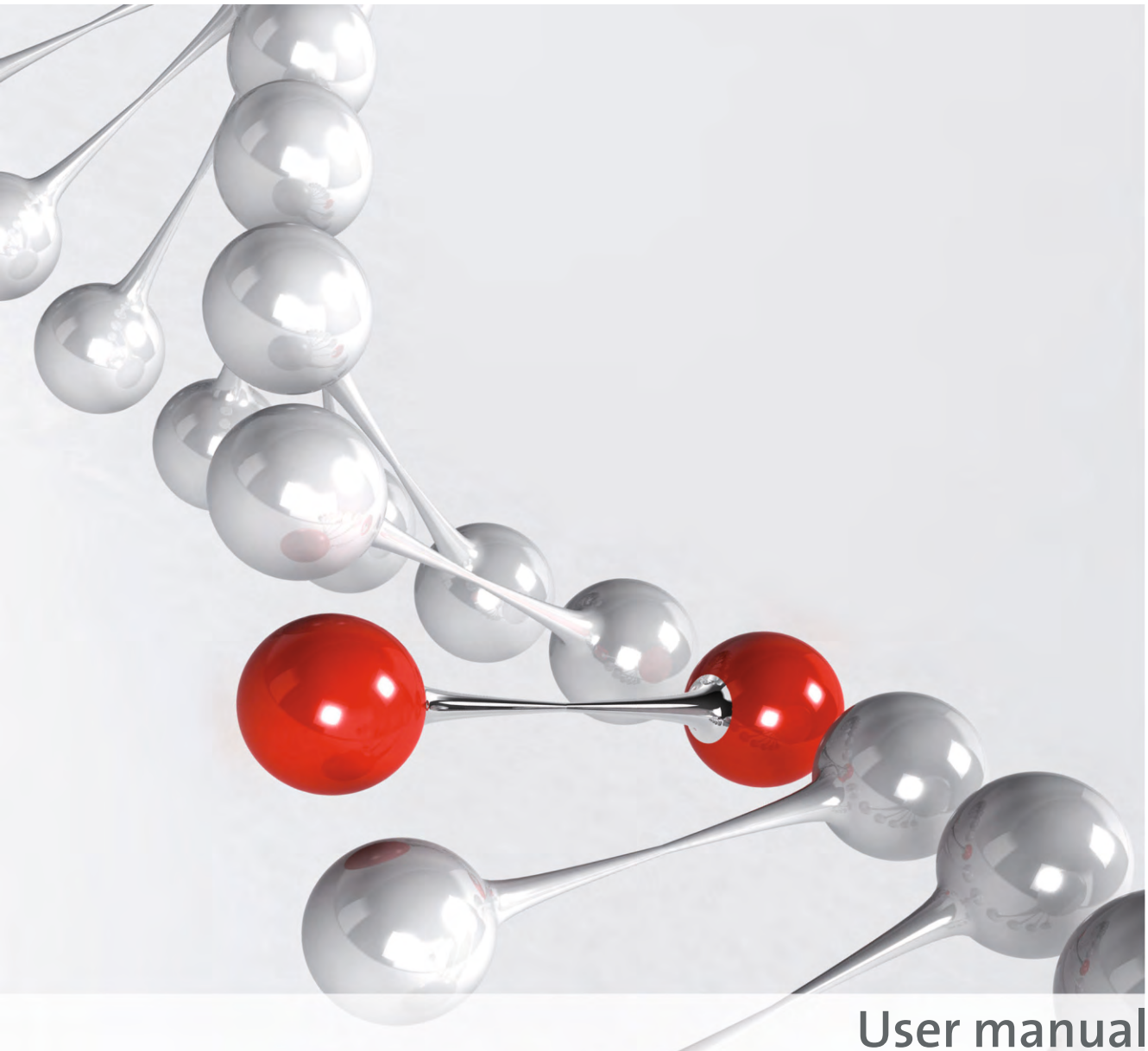

User manual
InviMag® Virus RNA Kit/ KF96

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

for automated purification of viral RNA from 200 µl serum, plasma, cell-free body fluids, rinse liquid from swab and stool sample or small tissue sample with magnetic beads

CE

IVD

REF 7443300x0

STRATEC Molecular GmbH, D-13125 Berlin

Instruction for InviMag® Virus RNA Kit/ KF96

The **InviMag® Virus RNA Kit/ KF96** combines the advantages of the innovative Invisorb® technology with easy handling of magnetic particles for a very efficient and reliable isolation of nucleic acids with high purity using the KF96 / KFflex96 instrument from ThermoFisher.

The **InviMag® Virus RNA Kit/ KF96** is designed for isolation and purification of pure viral RNA from fresh or frozen plasma, serum, cell-free body fluids as well as rinse liquid from swabs, stool samples or small tissue samples in a 96 well format. The interplay of the RNA extraction and purification chemistry provided by the **InviMag® Virus RNA Kit/ KF96** with KingFisher™ instruments was intensely tested and validated.

The RNA-binding magnetic particles are characterized by a high surface area, a uniform size distribution, good suspension stability and are therefore highly suitable for high throughput processing.

The kit is not for use with blood stains, cultured or isolated cells. The kit is also not suitable for isolating and purifying bacterial, fungal, or parasite DNA. The performance of the kit for isolating and purifying viral RNA from bone marrow or CSF has not been evaluated.

CE **IVD** CE-marked for compliance with EU IVD Directive 98/79/EC

Not for in vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

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Kit contents of InviMag® Virus RNA Kit/ KF96

	1 x 96 extractions	5 x 96 extractions
Catalogue No.	7443300100	7443300200
Lysis Buffer RV	60 ml	250 ml
Proteinase K	2 x 1.5 ml	10.5 ml
Carrier-RNA	2 ml	5x 2 ml
RNase Free Water	2 x 2 ml	15 ml
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 30 ml)	empty bottle (final volume 140 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 3x 160 ml)
Wash Buffer R2	50 ml (final volume 250 ml)	4 x 50 ml (final volume 4 x 250 ml)
Elution Buffer R	30 ml	120 ml
2.0 ml Deep Well Plate	4	20
KF96 Tip Combs	1	5
200 µl Elution Plate	2	2 x 5
Sealing Foils	2	10
1.5 ml Receiver Tubes	2 x 50 pieces	10x 50 pieces
Manual	1	1
Initial steps	Fill 30 ml 99.7% Isopropanol (molecular biologic grade) into the empty bottle Dilute **Proteinase K** by addition of 1.5 ml of ddH_2O, mix thoroughly until completely dissolving Dilute **Carrier-RNA** by addition of 2 ml **RNase Free Water**. Mix thoroughly until completely dissolving. Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer R1**. Add 200 ml of 96-100% ethanol to the bottle **Wash Buffer R2**. Mix thoroughly and always keep the bottles firmly closed!	Fill 140 ml 99.7% Isopropanol (molecular biologic grade) into the empty bottle Dilute **Proteinase K** by addition of 10.5 ml of ddH_2O, mix thoroughly until completely dissolving Dilute **Carrier-RNA** by addition of 2 ml **RNase Free Water**. Mix thoroughly until completely dissolving. Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer R1**. Add 200 ml of 96-100% ethanol to each bottle **Wash Buffer R2**. Mix thoroughly and always keep the bottles firmly closed!

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

Kit contents of InviMag® Virus RNA Kit/ KF96/ w/o plastic

	1 x 96 extractions	5 x 96 extractions
Catalogue No.	7443300150	7443300250
Lysis Buffer RV	60 ml	250 ml
Proteinase K	2 x 1.5 ml	10.5 ml
Carrier-RNA	2 ml	5 x 2 ml
RNase Free Water	2 x 2 ml	15 ml
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 30 ml)	empty bottle (final volume 140 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	50 ml (final volume 250 ml)	4 x 50 ml (final volume 4 x 250 ml)
Elution Buffer R	30 ml	120 ml
Sealing Foils	2	10
1.5 ml Receiver Tubes	2 x 50 pieces	10 x 50 pieces
Manual	1	1
Initial steps	Fill 30 ml 99.7%Isopropanol (molecular biologic grade) into the empty bottle Dilute **Proteinase K** by addition of 1.5 ml of ddH_2O, mix thoroughly until completely dissolving Dilute **Carrier-RNA** by addition of 2 ml **RNase Free Water**. Mix thoroughly until completely dissolving. Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer R1**. Add 200 ml of 96-100% ethanol to the bottle **Wash Buffer R2**. Mix thoroughly and always keep the bottles firmly closed!	Fill 140 ml 99.7% Isopropanol (molecular biologic grade) into the empty bottle Dilute **Proteinase K** by addition of 10.5 ml of ddH_2O, mix thoroughly until completely dissolving Dilute **Carrier-RNA** by addition of 2 ml **RNase Free Water**. Mix thoroughly until completely dissolving. Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer R1**. Add 200 ml of 96-100 % ethanol to each bottle **Wash Buffer R2**. Mix thoroughly and always keep the bottles firmly closed!
Plastic to be supplied by user (see order information)		
2.0 ml Deep Well Plate	4	20
KF96 Tip Combs	1	5
200 µL Elution Plate	2	10

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

Symbols

 Manufacturer

LOT Lot number

REF Catalogue number

 Expiry date

 Consult operating instructions

 Temperature limitation

 Do not reuse

 Humidity limitation

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

Storage

All buffers and kit contents of the **InviMag® Virus RNA Kit/ KF96,** except **dissolved Carrier-RNA, dissolved Proteinase K** should be stored at room temperature and are stable for at least 12 months.

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).

Proteinase K: Dissolved Proteinase K must be stored at 2 - 8 °C for up to two months. For longer storage –20 °C is recommended, freeze-thaw once only. So the dissolved Proteinase K is stable as indicated on the kit package.

Carrier RNA: Dissolved Carrier RNA must be stored at -20°C. So the dissolved mix is stable as indicated on the kit package.

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® Virus RNA 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® Virus RNA 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® Virus RNA 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:
from Germany: +49-(0)30-9489-2901/ 2910
from abroad: +49-(0)30-9489-2903/ 2907 or contact your local distributor.

Intended use

The **InviMag® Virus RNA Kit/ KF96** is designed for semi-automated extraction and purification of viral RNA from 1-96 samples using magnetic beads and the KF96 or KFflex96 workstation. The nucleic acid isolation procedure is suitable for routinely walk-away automated preparation of RNA from fresh or frozen plasma, serum, cell-free body fluids as well as rinsed liquid from swabs, stool samples or small tissue samples. For reproducible and high yields an appropriate sample storage is essential (see “Sampling and storage of the starting material”, page 10). Fresh or frozen plasma or serum from blood treated with EDTA or citrate (but ***not*** *with heparin*) from common blood collection systems can be used. All utilities (reagents and plastic ware) required for the preparation of viral RNA are provided by the **InviMag® Virus RNA Kit/ KF96** in different package sizes.

The procedure of the **InviMag® Virus RNA Kit/ KF96** is optimized for the isolation of viral RNA from up to 200 µl starting material. Samples with a smaller volume than 200 µl must be adjusted to a final sample volume of 200 µl using either RNase-free Water or 1x PBS prior to the start of the procedure.

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 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.

The kit is in compliance with EU Directive 98/79/EC on in vitro medical devices. However, the kit is not suitable for in vitro diagnostic use in countries where the EU Directive 98/79/EC is not recognized.

Product use limitation

The kit is not intended for use with blood stains and cultured or isolated cells. The kit is also not suitable for isolating and purifying bacterial, fungal or parasitic DNA. The performance of the kit in isolating and purifying viral RNA from bone marrow or CSF has not been evaluated.

The included chemicals are only useable once.

Changing the starting material or the 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 any 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. 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 lab and must not be used for purposes other than intended.

The kit and its content are not suitable for consumption.

Safety information

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

Avoid direct skin contact! Adhere the legal requirements for working with biological materials!

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® Virus RNA Kit/ KF96** procedures for residual risk materials. Therefore, liquid waste has to be handled and discarded accordingly to local safety regulations.

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

Lysis Buffer RV

Warning

H302-H312-H319-H332-H412-P280-P305+P351+P338-EUH032

Proteinase K

Danger

H315-H319-H334-H335-P280-P305+P351+P338

Wash Buffer R1

Warning

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

H302: Harmful if swallowed.
H312: Harmful in contact with skin.
H319: Causes serious eye irritation.
H332: Harmful if inhaled.
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.
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® Virus RNA Kit/ KF96

The **InviMag® Virus RNA Kit/ KF96** procedure is the ideal tool for an efficient extraction and purification of viral RNA from fresh or frozen plasma, serum, cell-free body fluids as well as rinsed liquid from swabs, stool samples or small tissue samples in a 96 well format using magnetic beads and the KF96 / KFflex96 instrument.

Starting material	Yield	Time for preparation
up to 200 µl serum or plasma up to 200 µl cell-free body fluids 200 µl rinse liquid from swab 50 mg stool sample small tissue samples	depends on the sample (source and storage)	about 45 min (with lysis)

The RNA isolation process is based on the interaction of nucleic acids with coated magnetic particles at adapted buffer conditions. The KF96 / KFflex96 instrument performs all steps of the RNA purification procedure automatically without any user intervention, except the initial prefilling of the plates. The procedure requires minimal interaction by the user, allowing safe handling of potentially infectious samples. Sample cross-contamination and reagent cross-over is effectively eliminated by this automated purification process.

The KingFisher™ workstation uses magnetic rods to transport the RNA-binding magnetic particles through the various purification phases: Lysis, binding, washing, drying and elution. The volume of buffers and other liquids necessary for RNA isolation is reduced to a minimum. Eliminating the direct liquid handling and increasing the automation level result in a fast, reliable and robust technique.

After a sample specific cell lysis in Lysis Buffer, optimal binding conditions are adjusted upon addition of Binding Buffer. The viral RNA are bound to the simultaneously added magnetic particles is separated by use of the magnetic rods controlled by the KingFisher instrument. Subsequent to three washing steps of the particle bound nucleic acids, the viral RNA is eluted in **Elution Buffer R**.

Due to the high purity, the eluted viral RNA is ready-to-use for a broad panel of downstream applications like Real-time PCR* (quantitative RT-PCR, like TaqMan® und Light Cycler® technologies) or array technologies.

The **InviMag® Virus RNA Kit/ KF96** is supplied with a comprehensive manual describing five protocols (page 15-17) for viral RNA purifications from different sample sources. For semi-automated isolation of viral RNA from up to 200 µl serum using magnetic particles in a single well format for up to 15 samples per run, STRATEC Molecular offers the **InviMag® Virus RNA Kit/KFmL** (see Ordering information, page 25).

For the isolation of viral RNA from a single sample STRATEC Molecular offers the **Invisorb® Spin Virus RNA Mini Kit** for use on a centrifuge or for 8–96 samples the **Invisorb® Virus RNA HTS 96 Kits** for use on a centrifuge, vacuum manifold or robotic station (see “Ordering information”, page 25). For further information please contact: +49 (0) 30 9489 2901, -2910 in Germany and from foreign countries: +49 (0) 30 9489 2903/ 2907 or your local distributor.

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

Product Validation

Samples

For all tests, dilutions of inactivated Influenza A virus (H1N1) pos.(++) cell lysates with unknown concentration were used in fresh or frozen plasma and in frozen urine as test system. Fresh plasma was stored at 4°C until use whereas frozen plasma and urine were stored at -20°C. Influenza A virus was purchased from "Gesellschaft für Biotechnologische Diagnostik mbH". The Influenza A samples were stored at -80°C.

PCR Inhibitor and Cross Contamination Test

To maximize the detection of any potential contamination event, positive (spiked plasma and urine samples with a dilution of 1:200 of Influenza A) and negative (only plasma) controls were arranged in alternating wells (see ***Fig 1*** "Checkerboard Pattern"). 200 µl starting material was used for the viral RNA extraction and 2.5 µl of the eluted RNA in a RT-PCR reaction.

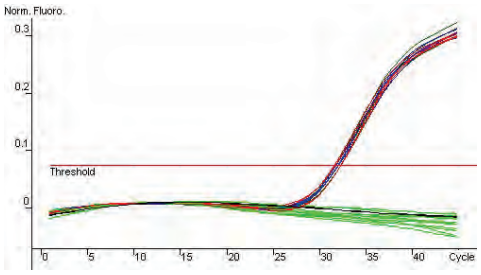
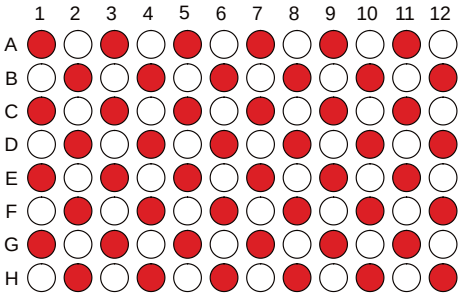


Fig. 2: Real-time RT-PCR results from positive samples from different starting materials (dark green – urine, blue - plasma 1, red – plasma 2) and 12 negative samples (green) arranged in Checkerboard. PTC (pink) and NTC (black) are also shown.

Fig. 1 **'Checkerboard Pattern'** utilized for the cross contamination analysis test. Samples (red) and NTCs (white) arranged in alternating wells.



Influence of different matrices on the extraction efficiency

To test the influence of different starting materials in regard to extraction efficiency, recovery and reproducibility a run using negative urine and two different plasma samples was performed. All samples were spiked with Influenza A virus in a dilution of 1:200.

Sample		mean Ct value	Std. deviation
	Urine	32.23	0.23
	Plasma 1	32.17	0.13
	Plasma 2	32.18	0.37
	PTC	31.94	0.45

Tab. 1: To show the high reproducibility the Ct mean value and the standard deviation for the samples in ***Fig 2*** are listed above.

The test shows, that the recovery, reproducibility and sensitivity are not influenced by the matrix of the sample. The tests demonstrate the good reproducibility and sensitivity of Influenza A RNA using different isolation systems from STRATEC Molecular.

Sampling and storage of starting material

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

Serum and plasma
After collection and centrifugation, of serum and/ or, plasma from blood (treated with anticoagulants like EDTA or citrate, *but **not** with heparin)*, synovial fluid samples or other cell-free body fluids, swabs as well as 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 in aliquots at -80°C. Frozen plasma or serum samples should not be thawed more than once. Multiple thawing and freezing cycles, before isolating the viral RNA, should be avoided because this may lead to denaturation and/or precipitation of proteins, resulting in reduced viral titers and yields. In addition, cryoprecipitates formed during freeze-thawing cycles may cause additional problems. If cryoprecipitates are visible, pellet them by centrifugation at app. 6.800 x *g* for 3 min. The cleared supernatant should be aspirated without disturbing the pellet and processed immediately. This step will not reduce viral titers.

Tissue
Best results are obtained with fresh tissue material or material that has been immediately flash frozen and stored at -20°C/-80°C. Repeated freezing and thawing cycles of stored samples should be avoided because this may lead to reduced yields. Use of poor quality starting material negatively influences the RNA yield too. The thawing process can be performed directly in **Lysis Buffer RV.**

Stool
Best results are obtained with fresh material. The collected stool sample can be stored at ambient temperature for at least 1-2 hours at RT, but the high content of DNases and RNases quickly leads to a DNA and RNA digestion and degradation. The sample should be quickly transferred into **Lysis Buffer RV** or can be stored frozen at -80°C for several weeks.

Swabs
The protocol works with fresh prepared swabs as well as with dried swabs. However, the purification process has not been validated for isolation of RNA from swabs which are stored in special storage buffers from other provider.

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 and procedure

The **InviMag® Virus RNA Kit/ KF96** procedure comprises following steps:

- Lysis of the virus particles in a 96 well plate format
- Binding of the viral RNA to the magnetic beads
- Washing and elimination of ethanol
- Elution of viral RNA

This manual contains five protocols (page 15-17).

Lysis
Samples are lysed in presence of **Lysis Buffer RV**, **Carrier-RNA** and **Proteinase K** using elevated temperatures.

Binding of the viral nucleic acids
After addition of **Binding Solution** and **SNAP Solution** to the lysate the viral RNA is bound to the magnetic beads.

Removing residual contaminants
Contaminants are efficiently removed using **Wash Buffer R1** and **R2**, while the viral RNA remains bound to the magnetic beads.

Elution
The viral RNA is finally eluted from the beads using **Elution Buffer R**. The eluted viral RNA is ready-to-use in different subsequent analysis such as Real-time PCR (quantitative RT-PCR, like Tasman® und Light Cycler® technologies) or array technologies.

Yield and quality of viral RNA

Different amplification systems vary in efficiency depending on the total amount of nucleic acid 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 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 any downstream analysis with NAT (nucleic acid tests) techniques such as examples qPCR, RT qPCR, LAMP, LCR. Diagnostic assays should be performed accordingly to the manufacturer's instructions.

Quantitative RT-PCR is recommended for determination of viral RNA 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 100-1000 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, correct quantities and quality. 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 because their use may lead to poor 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

Before starting a run, equilibrate all reagents to room temperature. Where necessary, gently mix and redissolve any precipitates by incubate at 30°C. Swirl gently to avoid foaming.
Lysis Buffer RV and **Elution Buffer R** are ready-to-use.
Add the required amount of distilled water (see Kit contents, page 4) to the **Proteinase K** and **Carrier-RNA** tube. Vortex for 5 s and store dissolved **Proteinase K** at -20°C if not used.

1 x 96 viral RNA-extractions:-
Add 30 ml **Isopropanol** into the empty bottle labeled with **"**Please fill empty bottle with 99.7% Isopropanol according to section "Kit Contents"
Dilute **Proteinase K** by addition of 1.5 ml of ddH_2O, mix thoroughly until completely dissolving!
Dilute **Carrier-RNA** by addition of 2 ml **RNase Free Water**. Mix thoroughly until completely dissolving.
Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer R1**.
Add 200 ml of 96-100% ethanol to the bottle **Wash Buffer R2**.
Mix thoroughly and always keep the bottles firmly closed!
5 x 96 viral RNA-extractions:
Add 140 ml **Isopropanol** into the empty bottle labeled with **"**Please fill empty bottle with 99.7% Isopropanol according to section "Kit Contents"
Dilute **Proteinase K** by addition of 10.5 ml of ddH_2O, mix thoroughly until completely dissolving!
Dilute **Carrier-RNA** by addition of 2 ml **RNase Free Water**. Mix thoroughly until completely dissolving.
Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer R1**.
Add 200 ml of 96-100% ethanol to each bottle **Wash Buffer R2**.
Mix thoroughly and always keep the bottles firmly closed!

*) Dissolved Carrier-RNA should be stored at -20°C. Avoid repeated freezing and thawing cycles because this may degrade the RNA and reduce the functionality of the Kit. Dividing diluted Carrier-RNA into aliquots is highly recommended.

Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Pipette and pipette tips
- Disposable gloves
- Distilled water
- Vortexer
- 96-100% ethanol
- Isopropanol *

*The **InviMag® Virus RNA Kit/ KF96** 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, molecular biology grade
Order no. A3928

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

Carrier-RNA

Carrier-RNA serves two purposes: It enhances the binding of viral nucleic acids to the magnetic beads, especially if there are only very few target molecules in the sample and reduces the risk of viral RNA degradation. If Carrier-RNA is not added a reduced viral RNA will be recovered.

Internal control (IC) / Extraction control

Internal controls (IC) from the PCR assay provider can be used as extraction controls. In that case add them to the Carrier-RNA. Never add extraction controls directly to the sample because if RNAses/DNases are present in the sample, the extraction control will get degraded.

Attention: Don´t add Internal Controls directly to the sample!

Important Note: **Using an internal control (IC)**

Using the InviMag Virus RNA Mini Kit /KF96 /KFflex96 in combination with commercially available amplification systems may require usage of an internal control (IC) to monitor the efficiency of sample extraction.

Internal control DNA or RNA (IC) must be combined with Carrier-RNA. For each sample a volume of 20 µl from the stock solution is added to the lysis mix.

Carrier-RNA is provided in tubes and must be adjusted to a final volume of 2000 µl stock solution by addition of RNase-free water. If internal controls for 96 samples must be added, please add 1 ml RNase-free water to the Carrier-RNA tube and add the amount of internal control required for 96 samples. Then adjust to a final volume of 2000 µl using RNase-free water. If you want to use internal extraction controls for less than 96 samples then adjust the Carrier-RNA with 1 ml of RNase-free water, take an aliquot of this solution (10 µl / sample + internal control) and adjust to a final volume of 20 µl / sample.

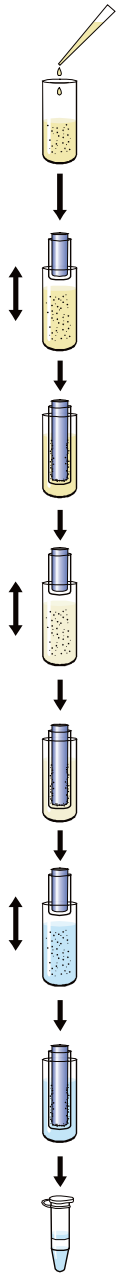
Scheme of the InviMag® Virus RNA Kit /KF96

Please read protocols prior the start of the preparation carefully

Add the required amount of RNase-free water or PBS to adjust the sample volume to 200 µl.
Transfer each sample in a corresponding well of the Binding Plate.

Prefill all plates of the KingFisher instrument with required buffers and the appropriate volumes.

Tip Plate:	Place in the KF96 Tip Comb for DW magnets on a Tip Plate*
Lysis Plate:	See respective lysis procedure (see page 15-17)
Washing Plate_1:	Add 750 µl **Wash Buffer R1**
Washing Plate_2:	Add 800 µl **Wash Buffer R2**
Washing Plate_3:	Add 800 µl **Wash Buffer R2**
Elution Plate:	Add 100 µl **Elution Buffer R (**same size as Tip Plate)



Lysis at elevated temperature (see protocols 15-17)

Addition of Binding Buffer and magnetic beads

Virus RNA binds to magnetic particles

Magnetic separation

3x Washing of the particle fixed viral RNA

Magnetic separation

Drying of viral RNA

Elution of viral RNA

Magnetic Separation

Pure viral RNA

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

Lysis procedures

For easier handling we recommend to prepare a master mix consisting of Proteinase K and Carrier RNA. When preparing the **Master Mix** it is recommended to use a volume of 5 % greater than that required.

Attention: *Please be aware, that you have to prepare the Master Mix shortly before carrying out the purifications adapted to the number of samples that will be processed.*
Longer incubation will decrease the activity of the Proteinase K

Preparation of a Master Mix

Number of samples	Amount of **Lysis Buffer RV**	Amount of **Carrier RNA**	Amount of **Proteinase K**
	400 µl / sample	20 µl / samples	20 µl / samples
8	3,4 ml	170 µl	170 µl
16	6,8 ml	340 µl	340 µl
24	10.0 ml	500 µl	500 µl
32	13,4 ml	670 µl	670 µl
40	16,8 ml	840 µl	840 µl
48	20.0 ml	1000 µl	1000 µl
56	23,6 ml	1180 µl	1180 µl
64	26,8 ml	1340 µl	1340 µl
72	30,2 ml	1510 µl	1510 µl
80	33,6 ml	1680 µl	1680 µl
88	37,0 ml	1850 µl	1850 µl
96	40.4 ml	2020 µl	2020 µl

Protocol 1: Extraction of viral RNA from serum, plasma, cell-free body fluids

Please read the instructions carefully and conduct the prepared procedure.

Important Note: *The protocol has been optimized for the isolation of viral RNA from 200 µl starting material. For samples with a smaller volume than 200 µl please adjust to a final volume of 200 µl using RNase-free water or 1x PBS.*

Transfer 200 µl sample carefully into an unused cavity of the Lysis Plate and add 400 µl **Lysis Buffer RV**, 20 µl **Proteinase K** and 20 µl **Carrier-RNA / IC Mix** (see page 133).

If all samples are transferred continue with the program (see “Starting a run”, page 17)

Protocol 2: Extraction of viral RNA from swab samples

Please read the instructions carefully and conduct the prepared procedure.

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a) Swab delivered in transportation media

Important: *If the swab is delivered in a stabilization medium, ensure that the medium is compatible with the STRATEC Molecular chemistry. For detailed information contact STRATEC Molecular: +49 30 9489 2901/ 2903/ 2907/ 2910.*

Transfer 200 µl from the stabilizing media including the dissolved viral particles to a free cavity of the Lysis Plate. Add 400 µl **Lysis Buffer RV**, 20 µl **Proteinase K** and 20 µl **Carrier-RNA/ IC Mix** (see page 133).

If all samples are transferred continue with the program (see "Starting a run", page 17).

b) Fresh or dried swabs without transportation media

Rinse each swab in 500 µl cooled water or 1x PBS and transfer a 200 µl aliquot to a free cavity of the Lysis Plate. Add with 400 µl **Lysis Buffer RV**, 20 µl **Proteinase K** and 20 µl **Carrier-RNA/ IC Mix** (see page 133).

If all samples are transferred continue with the program (see "Starting a run", page 17).

Protocol 3: Extraction of viral RNA from stool samples

Please read the instructions carefully and conduct the prepared procedure.

Pipet 400 µl ddH_2O into a 1.5 ml reaction tube (not provided). Add a glass stick to the stool sample and transfer the adherent sample (size of a lentil) in the prefilled 1.5 ml reaction tube. Close the tube and vortex vigorously until a homogenic suspension is formed.

Centrifuge the samples for 5 min at 12.000 x *g* (13.400 rpm). Carefully dip the pipette tip about 0.5 mm below the surface and transfer 200 µl from supernatant (prevent the aspiration of swimming particles) into the Lysis Plate. Add 400 µl **Lysis Buffer RV**, 20 µl **Proteinase K**, and 20 µl **Carrier-RNA / IC Mix** (see page 133).

If all samples are transferred continue with the program (see "Starting a run", page 17).

Protocol 4: Extraction of viral RNA from small tissue samples

Please read the instructions carefully and conduct the prepared procedure.

Homogenize 5–10 mg tissue samples in a mixer mill* or in liquid nitrogen using a mortar and pestle.

Note: *To maximize the final yield of viral RNA, a complete disruption of the tissue sample is important! For the disruption of starting material commercially available rotor-stator homogenizer or bead mills can be used. Alternatively, it is possible to disrupt the starting material using mortar and pestle with liquid nitrogen and grind the tissue sample to a fine powder.*

Add 200 µl ddH_2O or 1x PBS to each homogenate and resuspend it by pipetting up and down. Then transfer each sample into a free cavity of the Lysis Plate and add 400 µl **Lysis Buffer RV**, 20 µl **Proteinase K**, and 20 µl **Carrier- RNA / IC Mix** (see page 13).

If all samples are transferred continue with the program (see "Starting a run", page 17).

* The optimal disruption of tissue is important for obtaining maximum yields and purities of viral RNA. For high sample throughput it is recommended to use a mixer mill with a 96 well insert.

Protocol 5: Extraction of viral RNA from cell culture supernatant

Please read the instructions carefully and conduct the prepared procedure.

Transfer 200 µl from the cell culture supernatant (cell culture media) into an unused cavity of the Lysis Plate and add 400 µl **Lysis Buffer RV**, 20 µl **Proteinase K**, and 20 µl **Carrier-RNA IC Mix** (see page 133).

If all samples are transferred continue with the program (see "Starting a run", page 17).

Starting a run on a KF96 / KFflex96 instrument

Preliminary steps to process the samples onto the KingFisher™ 96/ KingFisher™ Flex 96 workstation

Important: *Before working with a KF instrument please carefully read the manufacturer's manual!*

1. Switch on the KF96 / KFflex96 instrument

   Tip Plate: Insert the Tip Plate in the KF 96 Tip Comb for DW magnets on a Tip Plate.

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

Note *If the prefilled plates are not used immediately, avoid evaporation of the prefilled buffer components by sealing the Deep Well Plates with a sealing foil or parafilm!*

Lysis Plate:	see respective lysis procedure (see page 15-17)
Washing plate_1:	Add 750 µl **Wash Buffer R1**
Washing plate_2:	Add 800 µl **Wash Buffer R2**
Washing plate_3:	Add 800 µl **Wash Buffer R2**
Elution Plate:	Add 100 µl **Elution Buffer R**

2. If a KF96 instrument is used, choose the program **"InviMag Virus RNA KF96"** on the display of the KingFisher 96 and press the "START" button. If a KFflex96 instrument is used, choose the program **"InviMag Virus RNA KFflex96"** and press the "START" button.
3. Insert the prefilled plates on the right position of the KingFisher machine surface by following the specifications printed on the display and confirm every loading step with the "Start" button.
4. If all prefilled plates are added to the KingFisher instrument press "Start" finally to initialize the assay file. The program will start with the lysis of the sample.

User intervention during the run

5. After lysis, a pause will automatically occur. At this step 260 µl **Binding Solution** and 20 µl **SNAP Solution** has to be added to sample containing cavities of the Lysis Plate.

Important: *Vortex the bottle with the **SNAP Solution** vigorously!*

6. Reinsert the plate (ensure correct plate orientation) and continue the program by pressing the "Start" button.

The following steps running automatically on the KF96 / KFflex96 instrument

1. Lysis of starting material

Automatically sample mixing and incubation at 65°C for 15 min in presence of **Lysis Buffer RV**, **Proteinase K** and **Carrier-RNA**

2. Program break for adding SNAP Solution and **Binding Solution**

After lysis, the program will pause and 260 µl **Binding Solution** and 20 µl **SNAP Solution** has to be added.

3. Binding of the viral RNA

Automatically sample mixing for 5 min. Magnetic bead separation. Moving of magnetic beads to Washing Plate_1.

4. First Washing

Automatically sample mixing for 1 min. Magnetic beads separation. Moving of the beads to Washing Plate_2.

5. Second Washing

Automatically sample mixing for 1 min. Magnetic beads separation. Moving of the beads to Washing Plate_3.

6. Third Washing

Automatically sample mixing for 1 min. Bead separation.

7. Drying

Drying the beads outside of Washing Plate_3 for 3.5 minutes.

8. Elution of the RNA

Incubation for 5 min. at elevated temperature while mixing is performed. Beads separation. Bead removal into the Washing Plate 3 (disposal).

Important Notes: *After finishing the extraction protocol, the Elution Plate contains the extracted viral RNA. Store the RNA at adequate conditions. We recommend transferring the extracted viral RNA into 1.5 ml reaction tubes (provided) for further storage and store them at -80°C.*

If the extracted RNA contains carry-over of magnetic particles, transfer the RNA into a 1.5 ml reaction tube, centrifuge at maximum speed for 1 min and transfer the RNA-containing supernatant into a new reaction tube.

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

For self-programming of the KF96 / KFflex96 instrument

Reagent info

Tip 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**
Lysis Buffer RV	400	-	Reagent
Proteinase K	20	-	Reagent
Carrier-RNA	20	-	Reagent
Sample	200	-	Sample

Wash Plate 1		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer R1	750	-	Reagent

Wash Plate 2		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer R2	800	-	Reagent

Wash Plate 3		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer R2	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]**
Binding Buffer + Beads	Adjust Binding Condition	280	-

Steps data

Tip1		96 DW tip comb	
	Pick-Up	Tip Plate	
	Lysis Step	Lysis Plate	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:15:00, Medium
		Heating temperature [°C]	65
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
	Adjust Binding Condition	Lysis Plate	
		Message	Add Binding+Beads
		Dispensing volume [µl]	280
	Reagent(s)	Name	Binding Buffer + Beads
		Volume [µl]	280
	Binding Step	Lysis Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	10
	Wash 1	Wash Plate 1	
	Beginning of step	Precollect	No
		Release time, speed	00:00:01, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	10
	Wash 2	Wash Plate 2	
	Beginning of step	Precollect	No
		Release time, speed	00:00:01, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	10

	Wash 3	Wash Plate 3	
	Beginning of step	Precollect	No
		Release time, speed	00:00:01, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	10
	Drying Step	Wash 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	00:05:00, Slow
		Heating temperature [°C]	60
		Preheat	Yes
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	10
	Bead Removal	Wash Plate 3	
		Release time, speed	00:00:30, Fast
	Leave	Tip Plate	

Troubleshooting

Problem	Probable cause	Comments and suggestions
low amount of extracted RNA	insufficient lysis	increase lyses time, but prevent too long lyses time because this also decrease yield reduce amount of starting material
	incomplete elution	Use a higher volume of **Elution Buffer R** but ensure that it is transferred into the right position
	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	elute the RNA in lower volume of **Elution Buffer R**
	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** and that the storage is correct
degraded RNA	incorrect storage of starting material	ensure that the storage of starting material was correct avoid multiple thawing and freezing cycles of the material
	old material	ensure that the starting material is fresh or stored at appropriate conditions (long-time storage at -80 C) avoid multiple thawing and freezing cycles 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 evaporation of ethanol
	salt carryover 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 RNA is brown 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 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 in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases have 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.

Electrophoresis tanks should be cleaned with detergent solution (e.g. 0.5% SDS), thoroughly rinsed with RNase-free water, and then rinsed with 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. It is possible to use chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.

- All buffers must be prepared with RNase-free ddH_2O.
- Change gloves frequently and keep tubes closed.
- Reduce the preparation time as much as possible.
- Keep isolated RNA on ice.
- Do not use 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 have been lysed.

This kit should only be used by personnel trained in *in vitro* diagnostic laboratory practice.

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® Virus RNA Kit/ KF96	1 x 96 preparations	7443300100
InviMag® Virus RNA Kit/ KF96	5 x 96 preparations	7443300200
InviMag® Virus RNA Kit/ KF96 w/o plastic	1 x 96 preparations	7443300150
InviMag® Virus RNA Kit/ KF96 w/o plastic	5 x 96 preparations	7443300250

KingFisher™ 96 and consumables

KingFisher 96, Magnetic Particle Processor,100-240V,50/60Hz		5400500
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
InviMag® Virus RNA Kit/ KFmL	75 preparations	2443110200
InviMag® Virus RNA Kit/ KFmL	300 preparations	2443110400

Related products	Package size	Catalogue No.
Invisorb® Spin Virus RNA Mini Kit	50 preparations	1040300200
Invisorb® Spin Virus RNA Mini Kit	250 preparations	1040300300
Invisorb® Spin Virus DNA Mini Kit	50 preparations	1040200200
Invisorb® Spin Virus DNA Mini Kit	250 preparations	1040200300

Possible suppliers for Isopropanol:

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

Applichem
2-Propanol, molecular biology grade
Order no. A3928

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

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