INVITEK
Molecular

USER MANUAL

InviMag® Virus RNA Kit/ KFmL

for semi-automated purification of viral RNA from plasma, serum, cell-free body fluids, rinse liquid from swabs, stool samples or small tissue samples with magnetic beads

CE IVD

REF 2443110400

Invitek Molecular GmbH · D-13125 Berlin

Instruction for InviMag® Virus RNA Kit/ KFmL

The **InviMag® Virus RNA Kit/ KFmL** 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 a high purity.

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

The **InviMag® Virus RNA Kit/ KFmL** for isolation and purification of pure 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 15 well format is designed for an optimal use on the KingFisher™ mL workstation from Thermo Scientific. The interplay of the RNA extraction and purification chemistry provided by the **InviMag® Virus RNA Kit/ KFmL** with the KingFisher™ instrument was intensely tested and validated.

The kit is neither suitable for isolation of viral RNA from whole blood, blood stains, cultured or isolated cells, tissue samples, bacteria, fungi, plants or viruses nor for purification of DNA.

CE IVD Compliance with EU Directive 98/79/EC on *in vitro* medical devices.

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

Content

Kit contents of InviMag® Virus RNA Kit/ KFmL

	300 preps
Catalogue No.	2443110400
Lysis Buffer RV	180 ml
Proteinase K	for 4 x 2 ml working solution
Carrier-RNA	for 6 x 1.2 ml working solution
RNase Free Water	15 ml
Binding Solution (fill with 98-100% Isopropanol)	empty bottle (final volume 80 ml)
MAP Solution A	7 ml
Wash Buffer R1	2 x 80 ml final volume 2 x160 ml
Wash Buffer R2	3 x 40 ml final volume 3 x 200 ml
Elution Buffer R	60 ml
Elution Tubes	6 x 50
KingFisher mL Tip Combs	60
KingFisher mL Tube Strips	300
Manual	1
Initial steps	Dilute each **Proteinase K** by addition of 2 ml ddH_2O, mix thoroughly until completely dissolving Dilute each **Carrier-RNA** by addition of 1.2 ml **RNase Free Water.** Mix thoroughly until completely dissolving. Fill 80 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer R1**. Add 160 ml of 96-100% ethanol to each bottle **Wash Buffer R2.** Mix thoroughly and always keep the bottles firmly closed!

Symbols

 Manufacturer

 Lot number

 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/ KFmL,** except **dissolved Carrier-RNA, dissolved Proteinase K** and **MAP Solution A** 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. Therefore, the dissolved Proteinase K is stable as indicated on the kit package.

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

MAP Solution A: The magnetic beads should be stored at 2-8°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

Invitek Molecular warrants the correct function of the **InviMag® Virus RNA Kit/ KFmL** for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform the applications as described in the manual, Invitek Molecular will check the lot and if Invitek Molecular investigates a problem in the lot, Invitek Molecular will replace the product free of charge. Invitek Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.
In accordance with Invitek Molecular's EN ISO 13485 certified Quality Management System the performance of all components of the **InviMag® Virus RNA Kit/ KFmL** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of the **InviMag® Virus RNA Kit/ KFmL** or other Invitek Molecular products, please do not hesitate to contact us. A copy of Invitek Molecular's terms and conditions can be obtained upon request or are presented at the Invitek Molecular webpage www.invitek-molecular.com.

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

Intended use

The **InviMag® Virus RNA Kit/ KFmL** is designed for semi-automated extraction and purification of viral RNA from 1-15 samples using magnetic beads and the KingFisher mL instrument. The nucleic acid isolation protocol is suitable for routinely 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 9). Fresh or frozen plasma or serum from blood treated with EDTA or citrate, (*not with heparin*) from common blood collection systems can be used. All utilities (reagents and plastic ware) necessary for preparation of viral RNA are provided by the **InviMag® Virus RNA Kit/ KFmL** in different package sizes.

The procedure of the **InviMag® Virus RNA Kit/ KFmL** is optimized for the isolation of viral RNA from up to 200 µl starting material. For samples of a smaller volume than 200 µl please adjust a total sample volume to 200 µl with RNase-free water or 1x PBS before starting an extraction protocol.

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

Product use limitation

The kit is not intended for blood samples, blood stains, cultured or isolated cells. The kit is also not suitable for isolating and purifying bacterial, fungal, or parasite nucleic acids. The performance of the kit in isolating and purifying viral nucleic acids from bone marrow or CSF has not been evaluated.

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, neither implied nor express.

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

Invitek 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 Invitek Molecular are subject to extensive quality control procedures (according to EN ISO 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to Invitek Molecular immediately upon detection thereof.

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

The product with its contents is unfit for consumption.

Safety information

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

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

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.invitek-molecular.com for each Invitek 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.

Invitek Molecular has not tested the liquid waste generated by the **InviMag® Virus RNA Kit/ KFmL** procedures for residual risk materials. Therefore, liquid waste has to be handled and discarded according to local safety regulations.

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

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
in USA : 1 – 800 – 535 – 5053

Product characteristic of the InviMag® Virus RNA Kit/ KFmL

The **InviMag® Virus RNA Kit/ KFmL** 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 rinse liquid from swabs, stool samples or small tissue samples in a 15- well format using magnetic beads and the KingFisher™ mL.

Starting Material	Yield	Time for Preparation	Ratio
up to 200 µl serum or plasma up to 200 µl cell-free body fluids 200 µl rinse liquid from swabs stool samples small tissue samples	depends on the sample (source and storage)	about 25 min (without lysis)	A_{260}:A_{280}: 1.7-2.0

The RNA isolation process is based on the interaction of nucleic acids with coated magnetic particles under adapted buffer conditions. The KingFisher™ mL performs all steps of the RNA purification procedure automatically without any user intervention. The procedure requires only minimal interaction by the user, thus allowing safe handling of potentially infectious samples. Sample cross-contamination and reagent cross-over is effectively eliminated by this automated purification process.

The KingFisher™ instruments use magnetic rods to move the RNA-binding magnetic particles through the various purification phases: binding-washing-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 results in a fast, reliable and robust technique. The overall efficiency speeds up the procedure.

After a sample specific cell lysis in the optimized **Lysis Buffer RV** in presence of **Proteinase K** and **Carrier-RNA**, optimal binding conditions are adjusted by addition of **Binding Solution.** The viral RNA bound to the simultaneously added magnetic particles is separated from solution by 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:

- RNA dot blots
- cDNA transcription
- Real-time PCR[1] (quantitative RT-PCR, like TaqMan® und Light Cycler® technologies)
- Array technologies

The results from downstream applications 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.

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

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

Sampling and storage of starting material

Best results are obtained using freshly extracted samples. As long as the samples are not shock frosted with liquid nitrogen or are incubated with RNase inhibitors or denaturing reagents, the RNA is not secured. Therefore, it is essential that samples are immediately flash frozen subsequent to the harvesting by using liquid nitrogen and are stored at -80°C. 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).

After collection and centrifugation, serum, plasma, from blood (treated with anticoagulants like EDTA or citrate, *but not with heparin)*, urine, 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 must not be thawed more than once. Multiple thawing and freezing cycles before isolating the viral RNA should be avoided because this leads to denaturation and precipitation of proteins, resulting in reduced viral titers and yields. In addition, cryoprecipitates formed during freeze-thawing cycles can cause problems. If cryoprecipitates are visible, they should be pelleted by centrifugation at app. 6.800 x *g* (9.000 rpm) for 3 min. The cleared supernatant should be aspirated without disturbing the pellet and be processed immediately. This step will not reduce viral titers.

Best results are obtained with fresh tissue material or material that has been immediately flash frozen and stored at -20°C or -80°C. Repeated freezing and thawing cycles of stored samples should be avoided because this leads to a reduced RNA yield. Use of poor quality starting material influences the RNA yield too. The thawing process could be proceed, e.g. directly in **Lysis Buffer RV.**

Invitek 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/ KFmL** procedure comprises following steps:

- lysis of the virus particles
- binding the viral RNA to the magnetic beads
- washing and elimination of ethanol
- elution of viral RNA

After lysis, the viral RNA binds to the magnetic beads whereas contaminations and enzyme inhibitors are efficiently removed during the following three wash steps and highly purified viral RNA is finally eluted in **Elution Buffer R**.

This manual contains 5 protocols (page 13-14).

Lysis
Samples are lysed under denaturing conditions at elevated temperatures in the presence of **Lysis Buffer RV**, **Proteinase K** and **Carrier-RNA**. In case of a large number of samples the preparation of a master mixture of a volume 5% greater than that required for the processing of all samples is recommended. Vortex the master mix carefully prior to use!

Binding of the viral nucleic acids
After adding **Binding Solution** and **MAP Solution A** to the lysate the viral RNA is bound to the surface of the magnetic beads.

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

Elution
The viral RNA is eluted from the beads using 100 µl **Elution Buffer R**. The eluted viral RNA is ready-to-use in different subsequent tests e. g. RNA dot blots, cDNA transcription, real-time PCR (quantitative RT-PCR, like TaqMan® 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 downstream analysis with NAT techniques, for examples qPCR, RT qPCR, LAMP, LCR. Diagnostic assays should be performed according 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 up to 1000 bases. The kit is not dedicated for applications using this kind of analysis.

Important notes

Important points before starting a protocol
Immediately upon receipt of the product, inspect the product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify Invitek Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the Invitek Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (see page 6). Do not use damaged kit components, since their use may lead to poor kit performance.

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

Internal control (IC) / Extraction control

Internal Controls (IC) from the PCR assay provider can be used as extraction controls if the fragments are longer than 100 bp. In this case, they have to be added after finalization of the lysis step. Alternatively, it can be mixed with the Carrier RNA.

Attention: Do not add directly these Internal Controls to the sample!

Preparing reagents and buffers

Before starting a run, bring all reagents to room temperature. Where necessary, gently mix and redissolve any precipitates by incubation at 30°C. Swirl gently to avoid foaming.

Lysis Buffer RV and **Elution Buffer R** are ready-to-use.

Add the required amount of ddH_2O (see Kit contents page 3) to the reaction tube with **Proteinase K**. Vortexing for 5 s. We recommend to prepare only as much Proteinase K as needed.

300 viral RNA-extractions:
Dilute each **Proteinase K** by addition of 2 ml ddH_2O, mix thoroughly until completely dissolving. Dilute each **Carrier-RNA** by addition of 1.2 ml **RNase Free Water.** Mix thoroughly until completely dissolving. Fill 80 ml 99.7% Isopropanol (molecular biologic grade) into the empty bottle. Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer R1**. Add 160 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, but repeated freezing and thawing cycles will degrade the RNA and reduce the functionality of the Kit. Dividing Carrier-RNA into aliquots and storage at –20°C is recommended.

Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Pipette and pipette tips
- Disposable gloves
- ddH_2O
- Vortexer
- 96-100% ethanol
- Isopropanol *

*The **InviMag® Virus RNA Kit/ KFmL** 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

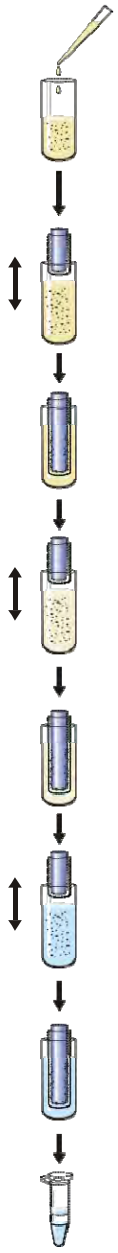
Carrier-RNA
Carrier-RNA serves two purposes. It enhances the binding of viral nucleic acids to the magnetic beads, especially if there are only very few target molecules present in the sample. Furthermore, the addition of large amounts of **Carrier-RNA** reduces the chance of viral RNA degradation in the **Lysis Buffer RV**. If **Carrier-RNA** is not added to the **Lysis Buffer RV**, this may lead to reduced viral RNA recovery.

Scheme of the InviMag® Virus RNA Kit/ KFmL

Please read protocols prior the start of the preparation carefully

Transfer each sample into a 1.5 ml reaction tube. Add the required amount of ddH_2O or 1x PBS to adjust the sample volume to 200 µl. Add 400 µl **Lysis Buffer RV**, 20 µl **Proteinase K** and 20 µl **Carrier-RNA**. Incubate for 10 min at 65°C on a thermoshaker.
Prefill all needed tubes stripes of the KingFisher mL with required buffers and appropriate volumes.

Tube A	Transfer the **lysed Sample** from above and add 260 µl **Binding Solution** and 20 µl **MAP Solution A**
Tube B	Add 800 µl **Wash Buffer R1**
Tube C	add 800 µl **Wash Buffer R2**
Tube D	Add 800 µl **Wash Buffer R2**
Tube E	Add 100 µl **Elution Buffer R**



After lysis, addition of 260 µl **Binding Solution** and 20 µl **MAP Solution A** is performed

Virus RNA binds to magnetic particle

Magnetic separation

3x Washing steps of the particle fixed RNA

Magnetic separation

Elution of viral RNA

Magnetic Separation

Pure viral RNA

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

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 of starting material. For samples which have a smaller volume than 200 µl please adjust up to a final volume of 200 µl with RNase-free water or 1x PBS.*

1. Transfer 200 µl of each sample carefully into 1.5 ml receiver tube.
2. Add 400 µl **Lysis Buffer RV**, 20 µl **Carrier-RNA** and 20 µl **Proteinase K.**
3. Incubate the sample at **65°C for 10 min** on a thermoshaker while continuously shaking (700 rpm).
4. After lysis, transfer the sample(s) into the Tube A of the KingFisher mL tube stripe and continue with the program (see “Starting a Run”, page 16).

Protocol 2: Extraction of viral RNA from swabs

Please read the instructions carefully and conduct the prepared procedure.

Swab delivered in transportation media

Important: *If the swab is delivered in a stabilization media, ensure that these media are compatible with the Invitek Molecular chemistry. For information, contact Invitek Molecular +49(0) 30 9489 2907.*

1. Some virus stabilizing media dissolve the viral particles. These can be used by transferring 200 µl carefully into a 1.5 ml reaction tube prefilled with 400 µl **Lysis Buffer RV**, 20 µl **Carrier-RNA** and 20 µl **Proteinase K**.
5. Incubate the sample at **65°C for 10 min** on a thermoshaker while continuously shaking (700 rpm).
2. After lysis, transfer the samples into the Tube A of the KingFisher mL tube stripe and continue with the program (see “Starting a Run”, page 16).

Fresh or dried swabs without transportation media

1. Rinse each swab with 500 µl precooled water or cooled PBS and use a 200 µl aliquot of the liquid for viral RNA extraction.
2. Transfer each sample carefully into a 1.5 ml reaction tube prefilled with 400 µl **Lysis Buffer RV**.
3. Add 20 µl **Carrier-RNA** and 20 µl **Proteinase K**
6. Incubate the sample at **65°C for 10 min** on a thermoshaker continuously shaking (700 rpm).
4. After lysis, transfer the samples to the Tube A of the KingFisher mL tube stripe and continue with the program (see “Starting a Run”, page 12).

Protocol 3: Extraction of viral RNA from cell culture supernatant

Please read the instructions carefully and conduct the prepared procedure.

1. Transfer 200 µl of the cell culture supernatant (cell culture media) into **Tube A** prefilled with 400 µl **Lysis Buffer RV**, 20 µl **Proteinase K**, and 20 µl **Carrier-RNA**.
2. Incubate the sample at **65°C for 10 min** on a thermoshaker continuously shaking (700 rpm).
3. If all samples are transferred continue with the program (see "Starting a Run", page 16).

Protocol 4: Extraction of viral RNA from stool samples

Please read the instructions carefully and conduct the prepared procedure.

1. Pipet 400 µl ddH_2O in a 1.5 ml reaction tube (not provided).
2. Add a glass stick to the stool sample and transfer the adherent sample (size of a lentil) into the prefilled 1.5 ml reaction tube.
3. Resuspend the sample in the prefilled water.
4. Close the tube and vortex each sample vigorously until it gets a homogenic suspension.
5. Centrifuge the sample for 5 min at 17.000 x *g* (15.000 rpm / e.g. Hettich Universal 30 RF).
5. Please carefully dip the pipette tip about 0.5 mm below the surface and transfer 200 µl supernatant (prevent the aspiration of swimming particles) into a 1.5 ml reaction tube prefilled with 400 µl **Lysis Buffer RV**, 20 µl **Carrier-RNA** and 20 µl **Proteinase K**
7. Incubate the sample at **65°C for 10 min** on a thermoshaker while continuously shaking (700 rpm).
6. After lysis, transfer the samples to the Tube A of the KingFisher mL tube stripe and continue with the program (see "Starting a Run", page 16).

Protocol 5: Extraction of viral RNA from small tissue samples

Please read the instructions carefully and conduct the prepared procedure.

1. Homogenize 5–10 mg tissue samples in a Mixer Mill* or using liquid nitrogen with a mortar and pestle.*
2. Add 200 µl ddH_2O or 1x PBS to each homogenate and resuspend the homogenate by pipetting up and down.
3. Transfer each sample carefully into a 1.5 ml reaction tube prefilled with 400 µl **Lysis Buffer RV**, 20 µl **Carrier-RNA** and 20 µl **Proteinase K**
8. Incubate the sample at **65°C for 10 min** on a thermoshaker continuously shaking (700 rpm).
4. After lysis, transfer the samples to the Tube A of the KingFisher mL tube stripe and continue with the program (see "Starting a Run", page 16).

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

Starting a Run

Preliminary Steps to process the samples onto the KingFisher™ mL System

Important: *Befor working with the KingFisher mL please carefully read the manufacturer´s manual!*

1. Switch on the KingFisher mL instrument

Note*: If the prefilled tube stripes will not be used immediately, please avoid evaporation of the prefilled buffer components by sealing the tubes with a sealing foil or with parafilm!*

Tube A: Transfer the lysed **Sample** and add 260 µl **Binding Solution** and 20 µl **MAP Solution A**

Tube B: Add 800 µl **Wash Buffer R1**

Tube C: Add 800 µl **Wash Buffer R2**

Tube D: Add 800 µl **Wash Buffer R2**

Tube E: Add 100 µl **Elution Buffer R**

2. Insert the filled KingFisher tube strips with the tube tray into the KingFisher instrument onto the correct position!
3. Place the KingFisher tip combs into the slots!
4. Initalize the KingFisher assay file "**InviMag Virus RNA KFmL**" by pressing the "Start" button

The following steps running automatically on the KingFisher mL System

1. Binding of the viral RNA

Automatically sample mixing for 5 min. MAP separation. Moving of MAP to Tube B.

2. First Washing

Automatically sample mixing for 1 min. MAP separation. Moving of the MAP to Tube C.

3. Second Washing

Automatically sample mixing for 1 min. MAP separation. Moving of the MAP to Tube D.

4. Third Washing

Automatically sample mixing for 1 min. MAP separation.

5. Drying

Drying the MAP outside of Tube D for 3.5 min.

6. Elution of the RNA

Incubation of the MAP into Tube E for 5 min. while mixing. MAP separation. The MAP will than automatically removed into the Tube D (disposal).

Important Notes*: After finishing the extraction protocol, the Tube E contains the extracted viral RNA. Store the RNA under adequate conditions. We recommend transferring the extracted RNA into the 1.5 ml reaction tubes for further storage and freeze the RNA at -80 °C.*

If the extracted RNA contains carry-over of magnetic particle, 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 tube.

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

For self-programming of the KingFisher™ mL instrument

Program "InviMag Virus RNA KFmL"

Name = InviMag Virus RNA Mini Kit/ KFmL
Protocol template version = 3.1
Instrument type = KFmL
Creator = STRATEC Molecular GmbH
Description = KFmL protocol (Thermo Electron) for isolation of viral RNA from clinical specimen
Plate layouts = King Fisher mL Tip Comb (1000µl stripes)

[PLATE LAYOUTS]

Plate type = KingFisher tubestrip 1000 µl
Plate change message = Press START
A:- volume = 640, name = lysed Sample
- volume = 260, name = Binding Solution
- volume = 20, name = MAP Solution A
B:- volume = 800, name = Wash Buffer R1
C:- volume = 800, name = Wash Buffer R2
D:- volume = 800, name = Wash Buffer R2
E:- volume = 100, name = Elution Buffer R

[Steps Data]

Binding Plate: (A)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Fast

Mixing parameters:
Mixing time [hh:mm:ss]: 00:05:00
Mixing speed: Medium

End of step:
Postmix: No
Collect count:4
Collect time [s]: 3

Wash1
Plate: (B)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Fast

Mixing parameters:
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Medium

End of step:
Postmix: No
Collect count: 3
Collect time [s]: 2

Wash 2
Plate: (C)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Fast

Mixing parameters:
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Medium

End of step:
Postmix: No
Collect count: 3
Collect time [s]: 2

Wash3
Plate: (D)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Fast

Mixing parameters:
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Medium

End of step:
Postmix: No
Collect count: 3
Collect time [s]: 2

Drying
Plate: (D)
Dry time [hh:mm:ss]: 00:03:30
Tip position: Outside well/tube

Elution Plate: (E)
Beginning of step:
Precollect: No
Release beads: Yes
Release time [hh:mm:ss]: 00:00:10
Release speed: Medium

Mixing parameters:
Mixing time [hh:mm:ss]: 00:05:00
Mixing speed: Slow

End of step:
Postmix: No
Collect count: 4
Collect time [s]: 3

Remove Beads
Plate: (D)
Release time [hh:mm:ss]: 00:00:30

Release speed: Fast

Troubleshooting

Problem	Probable cause	Comments and suggestions
low amount of extracted RNA	insufficient lysis	increase lyses time, but prevent long lyses times because this also decreases yields reduce amount of starting material
	incomplete elution	use a higher volume of **Elution Buffer R**, be sure you pipet the **Elution Buffer R** with the right amount to the right position
	low amount of MAP Solution A	mix **MAP Solution A** thoroughly before pipetting to the Stripe
low concentration of extracted RNA	too much Elution Buffer	elute the RNA with a lower volume of **Elution Buffer R**
	incorrect storage of starting material	ensure that the storage of starting material was correctly avoid repeated thawing and freezing cycles of the material
	incorrect Wash Buffers	make sure that the correct amount of ethanol is added to the **Wash Buffers** and stored correctly
degraded RNA	incorrect storage of starting material	ensure that the storage of starting material was correctly avoid multiple thawing of the material
	old material	ensure that the starting material is fresh or stored under appropriate condition (for long time storage at –80°C) avoid thawing and freezing of the material
RNA does not perform well in downstream-applications (e.g. RT-PCR or RT-PCR)	ethanol carryover during elution	increase drying time for removing of ethanol
	salt carryover during elution	check up the **Wash Buffers** for salt precipitates. If there are any precipitates, solve these precipitates by careful warming (up to 30°C) ensure that the **Wash Buffers** are at room temperature
low A_{260}:A_{280} ratio from UV measurement, eluted RNA is brown colored	small part of the magnetic particles are left in the elution	centrifuge down at full speed for 1 min and transfer supernatant to a new tube

Appendix

KingFisher™ Software 3.1

KingFisher Software 3.1 was used to create protocols for the KingFisher, KingFisher mL and KingFisher 96 instruments. Once a protocol has been created, the user can either transfer the protocol into the KingFisher workstation or run the protocol directly from the software. Directly run protocols are not stored in the workstation memory.

Note: *When creating the protocol using KingFisher instruments in combination with Microtiter Deep Well plates (e.g. Thermo Electron), it is essential to use the KingFisher software 3.1 for assay development as this software version includes the correct adjustments for this plate. It is highly recommended to use Thermo Microtiter Deep Well plates with KingFisher workstations to ensure the best purification result.*

PC Requirements

PC requirements for KingFisher Software 3.1

PC requirements	
Interface	Serial communication port via a RS-232 full duplex interface
Supported operating systems	Microsoft Windows 2000 Microsoft Windows XP Professional
Disk space	500 MB free disk space
Processor	Intel Pentium; 700 MHz recommended
Memory	220 MB RAM recommended
Serial ports available	1
Pointing device	Mouse or equivalent is necessary
CD-ROM drive	1
Monitor / color settings	SVGA monitor with at least 1024 x 768 resolution and at least a 16-bit color environment
Service packs installed	Microsoft Windows 2000: Service Pack 4 (or greater) Microsoft Windows XP Professional: Service Pack 2 (or greater)
Browser	Microsoft Internet Explorer 6.0 (or greater) installed

If you do not have the correct Service Packs installed, you can download them 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 everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases has to be reduced as much as possible. Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification is to be carried out.

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

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

Storage of viral 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/ KFmL	300 preparations	2443110400
Related products		
Invisorb® Spin Virus RNA Mini Kit	250 extractions	1040300200
InviMag® Virus RNA Kit/KF96	5 x 96 preparations	7443300200

Ordering information (KingFisher™ mL and consumables)

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

Possible suppliers for Isopropanol

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

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

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

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0062443110 V-01-2020