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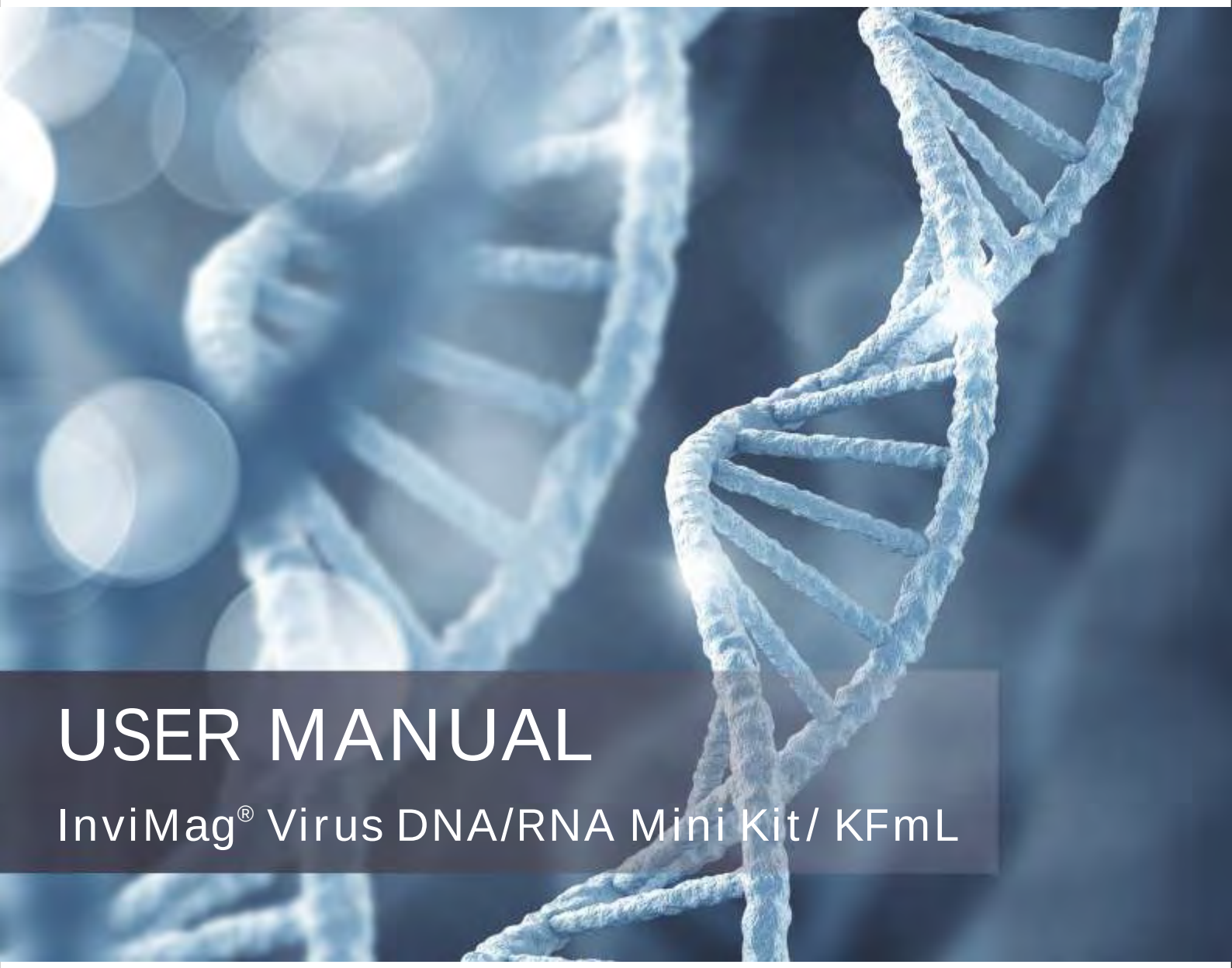

USER MANUAL

InviMag® Virus DNA/RNA Mini Kit/ KFmL

for semi-automated purification of viral DNA and RNA from up to 200 µl serum, plasma, cell culture supernatant and other cell-free body fluids, biopsy samples and swabs with magnetic beads

CE IVD

REF 2441150450

Invitek Molecular GmbH · D-13125 Berlin

Instruction InviMag® Virus DNA/RNA Mini Kit/ KFmL

The **InviMag® Virus DNA/RNA Mini Kit/ KFmL w/o plastic** is the ideal tool using a combination of RTP® technology and InviMag® technology for simultaneous isolation of high quality viral DNA and RNA from human and animal serum and plasma samples, cerebrospinal fluid, cell culture supernatants and other cell-free body fluids, like urine as well as from swabs or tissue biopsies for *in vitro* diagnostic purposes. Fresh or frozen plasma or serum from blood treated with anticoagulants like EDTA or citrate, *but not with heparin*. The customer convenient RTP® technology simplifies the process handling, reduces the handling steps with infectious material and allows process monitoring.

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

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

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.

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Kit contents of InviMag® Virus DNA/RNA Mini Kit/ KFmL w/o plastic

	300 extractions
Catalogue No.	2441150450
Extraction Tubes	6 x 50
MAP Solution A	7 ml
Binding Solution (fill with 98-100% Isopropanol)	empty bottle (final volume 140 ml)
Wash Buffer R1	2 x 80 ml (final volume 2 x 160 ml)
Wash Buffer R2	3 x 40 ml (final volume 3 x 200 ml)
Elution Buffer R	60 ml
Elution Tubes	6 x 50
Manual	1
Initial steps	Fill 140 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer R1**, mix thoroughly and always keep the bottle firmly closed! Add 160 ml of 96-100% ethanol to each bottle **Wash Buffer R2**, mix thoroughly and always keep the bottle firmly closed!
Plastic to be supplied by user (see order information)	
KingFisher mL Tip Combs	60
KingFisher mL Tube Strips	300

Symbols


Manufacturer

Lot number

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

Catalogue number


Expiry date


Consult operating instructions


Temperature limitation


Do not reuse


Humidity limitation

Storage

All buffers and kit contents of the **InviMag® Virus DNA/RNA Mini Kit/ KFmL w/o plastic,** except **MAP Solution A** should be stored at room temperature and are stable for at least 12 months. Store the Kit especially the Extraction Tubes in a dry environment, the Extraction Tubes must be protected from humidity.

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

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

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

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

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

Quality control and product warranty

Invitek Molecular warrants the correct function of the **InviMag® Virus DNA/RNA Mini Kit/ KFmL w/o plastic** 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 DNA/RNA Mini Kit/ KFmL w/o plastic** 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 DNA/RNA Mini Kit/ KFmL w/o plastic** 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 DNA/RNA Mini Kit/ KFmL w/o plastic** is designed for a simultaneous rapid and economical preparation of DNA and RNA from viruses from a wide range of clinical samples, using **RTP® technology**, magnetic beads, and the KingFisher mL workstation.
The whole process is based on a patented technology, the **InviMag® technology**, for isolation of viral DNA and RNA by binding the nucleic acid onto magnetic particles without chaotropic buffer components.
For reproducible and high yields, appropriate sample storage is essential. The purified DNA / RNA is of high quality and can be used for *in vitro* diagnostic 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.

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 neither validated for the isolation of genomic DNA from stool sample, dried blood stains, nor from bacteria, fungi, parasites or the purification of total RNA.

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.

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.

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

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 DNA/RNA Mini Kit/ KFmL w/o plastic** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

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

Extraction Tubes

Warning

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

Wash Buffer R1

Warning

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

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

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

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

Product characteristic of the InviMag® Virus DNA/RNA Mini Kit/ KFmL w/o plastic

Starting material	Yield	Time for preparation
up to 200 µl of fresh or frozen plasma, serum and cell free body fluids up to 200 µl cell culture supernatant up to 200µl of rinse liquid from swabs up to 10 mg biopsy samples	sensitive recovery realizing amplification with sensitive detection assays from staring material including minimum 100 copies per ml	about 20-25 min after lysis

The **InviMag® Virus DNA/RNA Mini Kit/ KFmL w/o plastic** is designed for a simultaneous rapid and economical preparation of viral DNA and RNA from a wide range of clinical samples, using a combination of RTP® technology, magnetic beads, and the KingFisher mL workstation.

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

The sample is lysed in an optimized lysis buffer. The lysates are transferred to the subsequent automatic purification procedure based on magnetic beads. The viral DNA and RNA bind to magnetic particles, followed by washing steps and a final elution. The procedure requires only minimal interaction by the user, allowing safe handling of potentially infectious samples. The procedure is designed to avoid sample-to-sample cross-contamination.
The purified high quality viral DNA and RNA is ready-to-use for subsequent downstream applications (see below) or can be stored at -20°C for subsequent use.

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

No toxic or hazardous chemicals like chaotropic components are used.

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

Sampling and storage of starting material

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

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

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

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

Tissue samples (biopsy material or frozen section)
Best results are obtained with fresh 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 low RNA yield. Use of poor quality starting material influences the RNA yield, too. The amount of purified RNA in the **Extraction Tube** procedure using up to 20 mg tissue sample, depends on kind of starting material. The thawing process could be proceed, e.g. directly in the **Extraction Tubes.**

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

Swabs
The protocol works with fresh prepared swabs or rinsed liquid from swabs or mouth brushes. Best results are obtained with fresh material or material that has been immediately frozen and stored at -20°C or -80°C.

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 DNA/RNA Kit/ KFmL w/o plastic** procedure comprises following steps:
- lysis of the virus particles in the prefilled Extraction Tube
- binding the viral NA to the magnetic beads
- washing of the magnetic beads and elimination of ethanol
- elution of the viral nucleic acids

After lysis, the viral NA bind to the magnetic beads, contaminations and enzyme inhibitors are efficiently removed during the following three wash steps and high-purified DNA/RNA is eluted in Elution Buffer R or water. This manual contains 4 protocols.

Lysis
Samples are lysed under denaturing conditions at elevated temperatures.

Binding of the viral nucleic acids
After adding **Binding Solution** and **MAP Solution A** to the lysate in the **Extraction Tube**, the nucleic acids are bound to the surface of the magnetic beads.

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

Elution
The nucleic acids are eluted from the beads using 100 µl **Elution Buffer R**.
The eluted nucleic acids are ready-to-use in different subsequent tests.

Yield and quality of viral DNA and / or 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 NA.

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. The amount of purified viral NA in the **InviMag® Virus DNA/RNA Mini Kit/ KFmL w/o plastic** procedure from plasma etc. depends on the sample type, sample source, transport, storage, and age. Quantitative RT-PCR is recommended for determination of viral NA yield.

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

Important notes

Important points before starting a protocol

Immediately upon receipt of the product, inspect the product and its components as well as the package for any apparent damages, 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 7). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps are carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard contaminated gloves immediately.
- Do not combine components of different kit, 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.

Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Pipette and pipette tips
- Disposable gloves
- Reaction tubes (1.5 ml or 2.0 ml)
- ddH_2O
- Vortexer
- 96–100% ethanol
- Isopropanol *

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

*** Possible suppliers for Isopropanol:**

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

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

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

Preparing reagents and buffers

300 viral NA-extractions:
Fill 120 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 always firmly closed
Add 140 ml of 96-100% ethanol to each bottle Wash Buffer R2, mix thoroughly and always keep the bottle firmly closed!

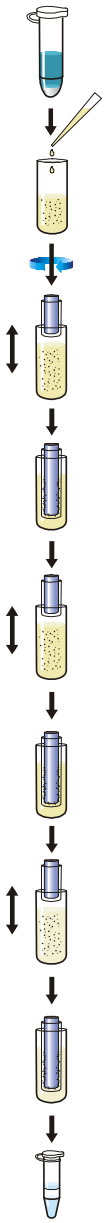
Important indications

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

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

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

Scheme of the InviMag® Virus DNA/RNA Kit/ KFmL



Please read protocols prior the start of the preparation carefully

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Add required amount of ddH_2O (200-400 µl) to the sample. Vortex for 10 s.
Place sample into a Thermomixer and incubate while continuously shaking for 15 min at 65°C, followed by an incubation for 10 min at 95°C.

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

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

Add approx. 450 µl of the lysed sample to Tube A

Viral NA binding to magnetic particle

Magnetic separation

Washing of the particle fixed viral NA

Magnetic separation

Elution of genomic DNA

Magnetic Separation

Pure viral NA

Protocol 1: Simultaneous isolation of total nucleic acids (viral DNA and RNA) from cell-free body fluids (serum, plasma, CSF, synovial, urine)

Please read the instructions carefully and conduct the prepared procedure.

Sample Lysis

1. Transfer 200 µl of the sample into the **Extraction Tube** and add 200 µl of ddH_2O Close the cap and mix by vortexing for 10 s**.**
2. Place the **Extraction Tube** into a Thermomixer and incubate while continuously shaking for 15 min at 65°C. During lysis, prefill all tubes with the required buffers and appropriate volumes (see page 17).
3. Place the Extraction Tube into another Thermomixer and incubate under continuously shaking for 10 minutes at 95°C.
   (These step leads to higher sensitivity for some viruses or some strains of viruses e.g. HIV or HCV)
4. After lysis, transfer the lysed sample into the Tube A of the KingFisher tube strip and add the 400 µl of **Binding Solution** and 20 µl **MAP Solution A**.

Note: ***Vortex the tube MAP Solution A vigorously before use!***

5. Start the assay file (see page 17).

Protocol 2: Simultaneous isolation of total nucleic acids (viral DNA and RNA) from cell culture supernatant

Please read the instructions carefully and conduct the prepared procedure.

Sample Lysis

1. Transfer 200 µl of the cell free cell culture supernatant (cell culture media) into the **Extraction Tube** and add 200 µl of ddH_2O. Close the cap and mix by vortexing for 10 s.
2. Place the **Extraction Tube** into a Thermomixer and incubate while continuously shaking for 15 min. at 65°C. During lysis, prefill all tubes with the required buffers and appropriate volumes (see page 17).
3. Place the Extraction Tube into another Thermomixer and incubate under continuously shaking for 10 min at 95°C.
   (These step leads to higher sensitivity for some viruses or some strains of viruses e.g. HIV or HCV.)
4. After lysis, transfer the lysed sample into the Tube A of the KingFisher tube strip and add the 400 µl of **Binding Solution** and 20 µl **MAP Solution A** (see also below).

Note: ***Vortex the tube MAP Solution A vigorously before use!***

5. Start the program, see instructions on page 17.

Protocol 3: Simultaneous isolation of total nucleic acids (viral DNA and RNA) from swabs or 200 µl rinse liquid

Please read the instructions carefully and conduct the prepared procedure

Sample Lysis

1a. Place the swab into the **Extraction Tube** and add 400 µl of ddH_2O.
Close the cap and mix by vortexing for 10 s**.**

1b. Transfer 200 µl of rinse liquid or of the stabilization media into the **Extraction Tube** and add 200 µl of ddH_2O. Close the cap and mix by vortexing for 10 s**.**

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

3. Place the Extraction Tube into another Thermomixer and incubate under continuously shaking for 10 min at 95°C.
(These step leads to higher sensitivity for some viruses or some strains of viruses e.g. HIV or HCV.)

4. After lysis, transfer the lysed sample into the Tube A of the KingFisher tube strip and add the 400 µl of **Binding Solution** and 20 µl **MAP Solution A** (see also below).

Note: ***Vortex the tube MAP Solution A vigorously before use!***

5. Start the program, see instructions on page 17.

Important Note: *To get maximum yield of viral nucleic acids it is essential to leave the swab during the complete lysis time into the reaction tube. It is possible to cut the shaft of the swab, so that you can close the cap of the Extraction Tube. It is also possible to do the lysis step with opened cap. The removing of the swab from the Extraction Tube ahead of time will be lead to a dramatically reduced final yield! After lysis time carefully squeeze out the swab on the wall of the tube and discard the swab.*

Protocol 4 : Simultaneous isolation of total nucleic acids (viral DNA and RNA) from tissue biopsy

Please read the instructions carefully and conduct the prepared procedure

Sample Lysis

1a. Transfer 1 mg – 10 mg of the tissue biopsy into the **Extraction Tube.**
Add 400 µl of ddH_2O. Close the cap and mix by vortexing for 10 s.

2. Place the **Extraction Tube** into a Thermomixer and incubate while continuously shaking for 15 min. at 65°C. Lysis time can be increased for up to 30 min. After a prolonged lysis a reduced yield and quality of some viral RNA species may occur.
After lysis, centrifuge the sample at max. speed for 1 min to spin down unlysed material and follow exactly the next step.
During lysis, prefill all tubes with the required buffers and appropriate volumes (see page 17).

3. Place the Extraction Tube into an other thermomixer and incubate while continuously shaking for 10 min. at 95°C.
(These step leads to higher sensitivity for some viruses or some strains of viruses e.g. HIV or HCV.)

4. After lysis, transfer the lysed sample into the Tube A of the KingFisher tube strip and add the 400 µl of **Binding Solution** and 20 µl **MAP Solution A**.

Note: ***Vortex the tube MAP Solution A vigorously before use!***

5. Start the program, see instructions on page 17.

Starting a run on the KFmL instrument

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

   KingFisher mL Tube Strip Setup

   Tube A**:** Filled with app. 450 µl of the lysed sample, 400 µl **Binding Solution** and 20 µl **MAP Solution A** after finishing the lysis step.

   It is important to mix the bottle with MAP Solution A by vigorously shaking or vortexing!

   Tube B: 800 µl **Wash Buffer R1**

   Tube C: 800 µl **Wash Buffer R2**

   Tube D: 800 µl **Wash Buffer R2**

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

2. Insert the prefilled KingFisher tube strips into the KingFisher instrument

3. Place the KingFisher tips onto the magnetic rack!

After these preliminary steps start the assay file "**InviMag Virus DNA-RNA KFmL**"

Important Notes:

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

The following steps will run automatically on the KingFisher™ mL

1. Binding of the DNA/RNA
Automatically sample mixing for 5 min. MAP separation. Moving of the MAP with bounded nucleic acids into the Tube B.

2. First Washing
Automatically sample mixing for 1 min. MAP separation. Moving of the MAP with bounded nucleic acids into the Tube C.

3. Second Washing
Automatically sample mixing for 1 min. MAP separation. Moving of the MAP with bounded nucleic acids into the Tube D.

4. Third Washing and Drying
Automatically sample mixing for 1 min. MAP separation. Drying the MAP with bounded nucleic acids outside of the Tube for 8 min. Moving of the MAP into the E wells.

5. Elution of the viral DNA and / or RNA
Incubation of the MAP into the Tube E for 10 min by mixing. MAP separation.
The MAP will than automatically removed into the B wells (disposal).

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

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

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

For self programming of the KingFisher™ mL system

[Protocol Properties]

Name = InviMag Virus DNA_RNA KFmL
Protocol template version = 3.1
Instrument type = KF96 / KFflex96
Kit name = InviMag Virus DNA-RNA Kit/ KF96/ KFflex96
Description = KF96 / KFflex96 protocol for Isolation of genomic DNA, viral DNA or RNA from swabs, tissue or suspension with the InviMag Virus DNA-RNA Kit/ KF96/ KFflex96

[Plate Layouts]

Binding plate

Plate type = KingFisher tub strip 1000µl
Reagents:
Name: Binding Solution
Volume [µl]: 400
Type: Reagent

Name: MAP A Solution
Volume [µl]: 20
Type: Reagent

Name: Lysed sample
Volume [µl]: 400
Type: Sample

Washing plate_1

Plate type = KingFisher tub strip 1000µl
Reagents:
Name: Wash Buffer R1
Volume [µl]: 800
Type: Reagent

Washing plate_2

Plate type = KingFisher tub strip 1000µl
Reagents:
Name: Wash Buffer R2
Volume [µl]: 800
Type: Reagent

Washing plate_3

Plate type = KingFisher tub strip 1000µl
Reagents:
Name: Wash Buffer R2
Volume [µl]: 800
Type: Reagent

Elution plate

Plate type = KingFisher tub strip 1000µl
Reagents:
Name: Elution Buffer R
Volume [µl]: 100
Type: Reagent

[Steps]

Binding (Plate: Virus DNA-RNA (A) – Binding)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Medium

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:05:00
Mixing speed: Slow

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

Washing_1 (Virus DNA-RNA (B) - Wash1)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Fast

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Medium

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

Washing_2 ((Virus DNA-RNA (C) – Wash2)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Fast

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Medium

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

Washing_3 (Virus DNA-RNA (D) – Wash3)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Fast

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Medium
End of step:
Postmix: No
Collect count: 3
Collect time [s]: 2

Drying (Virus DNA-RNA (D) – Wash3)
Dry time [hh:mm:ss]: 00:08:00
Tip position: Outside well / tube

Elution (Virus DNA-RNA (E) – Elution)
Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Medium

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:10:00
Mixing speed: Slow

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

Bead Removal (Virus DNA-RNA (D) – Wash3)
Release time [hh:mm:ss]: 00:00:30
Release speed: Fast

Troubleshooting

Problem	Probable cause	Comments and suggestions
low amount of extracted DNA/RNA	insufficient lysis	increase lyses time, but prevent too long lyses tome because this also decrease yield reduce amount of starting material
	incomplete elution	take higher volume of **Elution Buffer D**, be sure you pipet the **Elution Buffer D** with the right amount to the right position
	low amount of MAP Solution A	mix **MAP Solution A** thoroughly before pipetting to the KingFisher tube
low concentration of extracted DNA/RNA	too much Elution Buffer	elute the DNA with lower volume of Elution Buffer D
	incorrect storage of starting material	ensure that the storage of starting material was correctly avoid thawing of the material
degraded or sheared DNA/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 under appropriate condition (for long time storage at –20°C)! avoid thawing and freezing of the material old material often contains degraded DNA
DNA/RNA does not perform well in downstream-applications (e.g. real-time PCR or PCR)	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 ensure that the **Wash Buffers** are at room temperature
low A_{260}:A_{280} ratio from UV measurement, eluted DNA is brown colored	small part of the magnetic particles are left in the elution	centrifuge down at full speed for 1 min and transfer supernatant to a new tube

Appendix

KingFisher™ Software 3.1

The KingFisher Software 3.1 was used to create assay files for the KFmL, KF96 and KFflex96 instruments. The respective assay file can either be transferred onto the KingFisher workstation or be started directly from within the BindIt software. Keep in mind that directly run assay files are not stored in the workstation memory!

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 3.1 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 KF96 / KFflex96 workstations to ensure the best purification result.*

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 DNA

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

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

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

Drying, dissolving and pipetting DNA
Avoid overdrying genomic DNA after ethanol precipitation. It is better to air dry than to use a vacuum, although vacuum drying can be used with caution.
Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

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

General notes on handling viral 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 DNA/RNA Mini Kit/ KFmL w/o plastic	300 preparations	2441150450
Related products		
RTP® DNA/RNA Virus Mini Kit	250 extractions	1040100300

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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