

USER MANUAL

InviMag[®] Universal Kit/ STARlet

for automated purification of DNA (genomic, bacterial, mitochondrial and viral) as well as viral RNA from 200 µl clinical samples with magnetic beads

Instruction for InviMag® Universal Kit/ STARlet

The **InviMag® Universal Kit/ STARlet** combines the advantages of the innovative InviMag® technology with easy handling of magnetic particles of high purity in combination with the Hamilton® STARline platform for a very efficient and reliable isolation of nucleic acids in a high purity. The kit is the ideal tool for walk-away automated isolation and purification of total (genomic, bacterial) DNA and/or viral DNA/ RNA from up to 200 µl sample volume.

The interplay of the nucleic acid extraction and purification chemistry provided by the **InviMag® Universal Kit/ STARlet** was intensely tested and validated. The nucleic acid binding particles are characterized by a high surface area, uniform size distribution, good suspension stability and therefore are highly suitable for high-throughput processing.

Due to the high purity of the derived eluates, the isolated nucleic acids are ready-to-use in a broad spectrum of downstream applications or can alternatively be stored at -20°C/ -80°C for subsequent use.



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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The Invisorb® technology is covered by patents and patent applications: US 6,110,363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

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Kit contents of InviMag® Universal Kit / STARlet

Note: 2 ml Sarstedt Screw Tubes are necessary for preparation!
Buffer Volumes and Plates are calculated for the use of whole Plates!

	24 x 96 extractions
Catalogue No.	7450330400
Lysis Buffer HLT	6 x 120 ml
Proteinase K	48
Carrier RNA	48
RNase free Water	3 x 60 ml
SNAP Solution	48 x 1.6 ml
Binding Solution	empty bottle (final volume 1000 ml)
Wash Buffer HLT	3 x 600 ml (final volume 3 x 1000 ml)
Wash Buffer II	5 x 300 ml (final volume 5 x 1000 ml)
Elution Buffer M	6 x 160 ml
2.0 ml Collection Plate	72
Elution Plate L	24
Sealing Foils	72
Initial steps	<p>Dilute each tube Proteinase K by addition of 1300 µl RNase free Water, mix thoroughly.</p> <p>Dilute each tube Carrier RNA by addition of 1000 µl RNase free Water, mix thoroughly.</p> <p>Fill 1000 ml >99.7% Isopropanol (molecular biologic grade) into the empty bottle</p> <p>Add 400 ml >99.7% Isopropanol to each Wash Buffer HLT.</p> <p>Add 700 ml of 96-100% ethanol to each Wash Buffer II. Mix thoroughly and always keep the bottle firmly closed!</p>

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® Universal Kit/ STARlet**, except **dissolved Proteinase K** and **dissolved Carrier RNA** 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.

Wash Buffers charged with ethanol or isopropanol 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® Universal Kit/ STARlet** 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® Universal Kit/ STARlet** 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® Universal Kit/ STARlet** 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® Universal Kit/ STARlet** has been designed for fully automated extraction and purification of viral DNA/ RNA, genomic DNA, bacterial DNA from 96 samples per run by using magnetic beads with the STARlet system from Hamilton. Common collection tubes can be used to assemble a set of samples. All utilities (reagents and plastic ware beside components obtainable by Hamilton Inc. (as filter-tips and reagent trays) necessary for preparation of total NA are provided by the **InviMag® Universal Kit/ STARlet**.

The nucleic acid isolation protocol is suitable for routinely walk-away automated preparation of DNA and RNA from 200 µl of fresh or frozen sample material like human blood*, serum*, plasma*, cerebrospinal fluid, cell culture supernatants and other cell free body fluids, rinsed liquid from swabs, urine, supernatant from stool suspension, BAL, sputum etc. Targets are nucleic acids from viruses, genomic DNA from blood and bacterial DNA. For efficient extraction an appropriate sample storage is essential (see "Sampling and storage of the starting material", page 13).

*) Fresh or frozen blood, plasma or serum treated with EDTA or citrate, (*not with heparin*)

The whole process is based on the patented **InviMag®** technology, which relies on binding of the nucleic acids by magnetic particles. The procedure only requires minimal user interaction (prefilling of the plates), allowing safe handling of potentially infectious samples.

The DNA/ RNA purification procedure is performed with minimal user intervention, except the initial loading of the system and plate preparation. This allows safe handling of potentially infectious samples.

THE PRODUCT IS INTENDED 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

No guarantee in operability is issued with deviating starting materials, sample type or change in the procedure. The included chemicals are only useable once.

Deviation of starting material or the process sequence may lead to inoperability; therefore, neither a warranty nor guarantee in this case will be given, neither implied nor expressed.

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 waste generated by the **InviMag[®] Universal Kit/ STARlet** procedure for residual infectious materials. Contamination of the waste with residual infectious materials is unlikely, but cannot be excluded completely. Therefore, the waste has to be considered infectious and should be handled and discarded according to local safety regulations.

European Community risk and safety phrases for the components of the **InviMag[®] Universal Kit/ STARlet**, to which they apply, are listed below.

Lysis Buffer HLT



Warning

H302-315-319-P280-305+351+338

Proteinase K



Danger

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

H302: Harmful if swallowed.

H315: Causes skin irritation.

H319: Causes serious eye irritation.

H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled.

H335: May cause respiratory irritation.

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.

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 characteristics of the InviMag® Universal Kit/ STARlet

The **InviMag® Universal Kit/ STARlet** is the ideal tool for an efficient and fully automated DNA extraction and purification from fresh or frozen whole blood samples or DNA/ RNA from fresh or frozen plasma/serum using magnetic beads in combination with the STARlet, a Hamilton® robotic platform. The **InviMag® Universal Kit/ STARlet** provide several key features: high recovery rates for low virus titers and nucleic acid amounts, high reproducibility, complete automation for different sample types without the need of additional manual preparations, direct processing of primary tubes and an optimal utilization of 96 well plate capacity. These features reduce the risk for operator errors while increasing process reproducibility and maintaining flexibility of samples types. Sample cross-contamination and reagent cross-over is effectively eliminated.

Starting Material	Yield	Time for preparation
200 µl fresh or frozen blood*, 200 µl fresh or frozen plasma*/ serum 200 µl cell free body fluids (urine) 200 µl rinsed liquid from swab 200 µl transport media (Surepath, Thinprep) 200 µl supernatant from stool suspension 200 µl from liquid sputum, BAL, tracheal secrete *blood, plasma: EDTA /Citrate stabilized, but no heparin	depending on sample (storage and source) <u>Note:</u> The added Carrier-RNA will account for some of the eluted nucleic acid(s). Quantitative (RT)- PCR is recommended for determination of the viral RNA or DNA yield	about 180 min

The Starlet instrument uses an 8-channel pipettor to transfer samples and buffers and a magnetic tool to separate the DNA/ RNA bound to magnetic particles during the various extraction phases: lysis-binding-washing-elution. The automated purification process results in a fast, reliable and robust technique.

After a sample specific lysis, using **Lysis Buffer HLT** and **Proteinase K (Lysozyme)** too if required), binding conditions are adjusted upon addition of **Binding Solution**. The genomic DNA and/or viral DNA/ RNA bind to the added magnetic particles (**SNAP Solution**) and is separated from the solution by magnetic tool controlled by the STARlet system. Subsequent to three washing steps of the particle bound nucleic acids, the nucleic acids are finally eluted in **Elution Buffer M**.

The instrument provides two run files for using the **InviMag® Universal Kit/ STARlet**:

1. Blood samples are included in the sample pool for a run (Invimag_Universal_Blood assay)
2. No blood samples are included (this process needs less pipette tips, see page XX)
(Invimag_Universal assay).

Due to the high purity, the eluted nucleic acids are ready-to-use in a broad panel of downstream applications like:

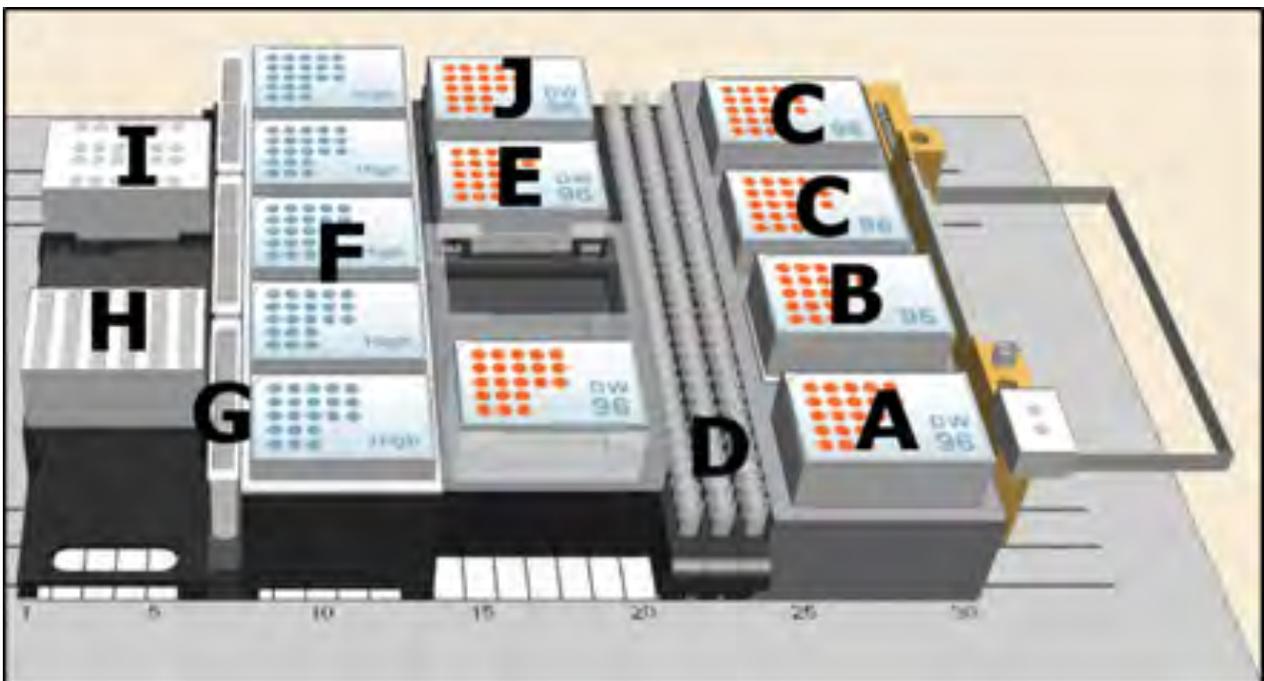
- PCR, Real-time PCR, PCR, qPCR
- HLA Typing
- Southern Blot

Equipment characteristics of the STARlet platform

The **STARlet** uses air displacement technology, which is analogous to a hand held pipette. Technological innovations implemented on the **STARlet** include independent and asymmetric positioning of pipetting channels, precise tip attachment and unrivalled dual liquid level detection. These innovations provide a wide volume range and quality pipetting. The **STARlet** meets the strictest requirements regarding positional accuracy, precision and flexibility. With unique features you can be assured that your application will be automated with the best process security, reliability and throughput available.

Carrier placement

Place the Tip-Rack-Carrier (F) as a barrier between the “clean” and “dirty” areas on the machine. Clean Reagent trays are placed on the left side (G, H and I) of the machine. This positioning avoids sample-contamination. That is why the samples (primary tubes) are placed at position D.



Carrier Placement, Screenshot of the actual STARlet Venus Software screen.

Position A (HeaterShaker), B (Magnetic Tool), C (Waste Plates) and E (Elution Plate) comprise the 5 needed Deep Well Plate positions. Position J is needed for pipettor mixing of the beads as a defined positioning on the HeaterShaker is not possible.

This layout has an additional vacuum station (visible in front of E) which is not needed for the **InviMag® Universal Kit/ STARlet**.

Principle and procedure

The **InviMag® Universal Kit / STARlet** procedure comprises following steps:

- Sample preparation if needed
- Lysis and protein digestion
- Binding of the DNA/ RNA to the magnetic beads
- Washing the bead bound DNA/ RNA
- Elimination of alcohol
- Elution of DNA/ RNA

After lysis the DNA/ RNA binds to the magnetic beads, contaminations and enzyme inhibitors are efficiently removed during the following wash steps and purified DNA/ RNA is eluted in **Elution Buffer M**.

Pretreatment: please check in the specific section in the protocol

Procedure

Lysis

Samples are lysed in a Deep Well Plate at elevated temperatures in the presence of **Lysis Buffer HLT** and **Proteinase K/ Carrier RNA**

Binding of the genomic DNA/ RNA

After addition of **Binding Solution** and **SNAP Solution (magnetic beads)** to the lysate, the DNA/ RNA is bound to the surface of the beads.

Removing residual contaminants

Contaminants are efficiently washed away while the DNA/ RNA remains bound to the magnetic beads.

Elution

The DNA/ RNA is finally eluted in **Elution Buffer M**. The eluted DNA/ RNA is ready for use in different subsequent downstream applications e.g. for PCR amplification, digestion with restriction enzymes, Southern hybridizations, HLA typing etc.

Yield and quality of genomic DNA from blood

The amount of purified genomic DNA in the **InviMag® Universal Kit/ STARlet** procedure from whole blood depends on the leucocytes content, the sample source, transport, storage, and age.

Typically, a volume of 200 µl of a whole blood sample from a healthy individual with a white blood cell content - ranging from 3×10^6 to 1×10^7 cells/ ml - will yield at least 3 µg of genomic DNA. The typical yield usually expected from the **InviMag® Universal Kit/ STARlet** is about 3 - 6 µg DNA. If a whole blood sample is mixed with anticoagulant containing buffer solutions the overall leukocyte concentration decreases and the yield of the DNA extraction procedure is reduced.

Yield and quality of pathogen DNA/ RNA

The amount of purified pathogen DNA/ RNA in the **InviMag® Universal Kit/ STARlet** procedure depends on the sample type, the virus and bacteria content, sample source, transport, storage, and age.

Yield and quality of isolated pathogen DNA/ RNA are suitable for any molecular detection system.

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 PCR**, 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 DNA or RNA yield.

Note: *If beads are visible in the eluate, transfer the eluate to a new reaction tube and centrifuge for 1 min at maximum speed (e.g. 13000 rpm).*

*) 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 Invitex Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the Invitex 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.

- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard gloves if they get contaminated.
- 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
- This kit should only be used by trained personnel.

Preparing reagents and buffers

24 x 96 extractions:

Dilute each tube **Proteinase K** by addition of 1300 µl RNase free Water, mix thoroughly

Dilute each tube Carrier RNA by addition of 1000 µl RNase free Water, mix thoroughly

Fill 1000 ml >99.7% **Isopropanol** (molecular biologic grade) into the empty bottle

Add 400 ml >99.7% Isopropanol to each **Wash Buffer HLT**.

Add 700 ml of 96-100% ethanol to each **Wash Buffer II**. Mix thoroughly and always keep the bottle firmly closed!

Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Conductive Tips with Filter, 1ml***
- Disposable gloves
- ddH₂O
- Vortex
- 96-100% Ethanol
- >99.7% Isopropanol* (molecular biological grade)
- Lysozyme**
- 2 ml Micro tubes (Sarstedt: REF: 72.609, 2 ml PP)

*The **InviMag® Universal Kit/ STARlet** 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

**Possible supplier for Lysozyme:

Fa. Applichem

Lysozyme, freeze dried

Order -no. A4972.0010

***Supplier for conductive tips

Hamilton Robotics

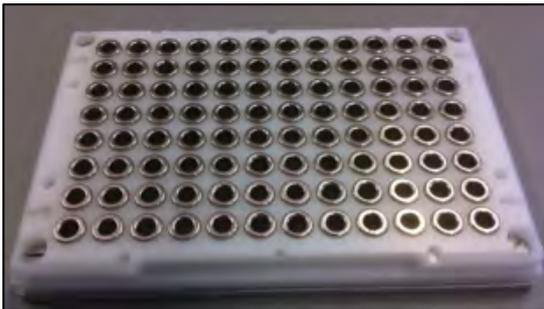
Conductive Tips with Filter, 1ml

Order no: 235905 High Volume CO_RE Tips – Case of 3840 tips_Blister Pack

Hamilton equipment to be supplied by the customer

The **InviMag® Universal Kit/ STARlet** has been developed on a Hamilton STARlet platform with 8 channels. 4 channels may work though the assay will need more time and the package needs to be customized.

- STAR or STARlet platform with 8 channels
- 3 big reagent trays (187297)
- 3 small reagent trays (182703)
- reagent carriers for the trays
- a 2-mm Heater-Shaker with an Universal Adapter Plate
- Plate-holders for 5 plates
- Magnetic-Ring Stand (96 Well) from Ambion/Invitrogen (AM10050)



- Multiflex tube / cup module, 188048
- Tip-Rack-Carrier for at least three Tip-Racks (one will be reused for supernatant removal)
- CO-RE gripper with attachment for waste block; 188066APE
- Conductive Filter Tips, see page 11

Sampling, storage and preparing of starting materials for processing on the Hamilton® system

Please read the instructions carefully and conduct the prepared procedure.

Important Note: The protocol has been optimized for the isolation of total DNA and viral RNA from up to 200 µl of liquid samples.

Sampling and storage

For reproducible and high yields appropriate sample storage is essential. Yields may vary from sample to sample depending on factors such as health of the donor, sample age, kind of sample, transport and storage conditions.

Blood: Best results are obtained using fresh blood samples. Blood samples (stabilized with EDTA or citrate but not heparin) can be stored at room temperature (18 - 25°C) for 2 - 3 hours. For short time storage (up to 24 h) samples should be stored at 2-8°C. For long term storage, we recommend to freeze the samples at -20°C or -80°C. Avoid multiple thawing and freezing cycles of the sample(s) before isolating the DNA/ RNA. Various different primary tubes, blood collection system (e.g. Sarstedt, Greiner) and anticoagulants (except heparin) can be used to collect blood samples for the InviMag® Universal Kit/ STARlet procedure

Serum and plasma: After collection and centrifugation, serum, 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 2-8°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 before isolating the viral DNA/ RNA should be avoided. It leads to denaturation and precipitation of proteins, resulting in reduced viral titers and therefore reduced yields of viral nucleic acids. In addition, cryoprecipitate formed during freeze-thawing could make problems. If cryoprecipitate is visible, after vortexing they should be pelleted by centrifugation at app. 6.800 x g for 3 minutes. The cleared supernatant should be aspirated, without disturbing the pellet and processed immediately. This step will not reduce viral titers.

Swabs: The protocol works with rinsed liquid from fresh prepared swabs as well as with dried swabs as well as from pretreated fresh saliva. The protocol has not been validated for isolation of RNA from swabs which are stored under special storage buffers of other providers.

Cultivated bacteria or bacterial suspension(s): Bacteria have to be pelleted after cultivation and suspended in defined conditions; best results are obtained with fresh material.

Processing of bacterial samples: The kit was validated with *Bacillus subtilis* spiked cell-free medium. To perform a quantitative extraction of bacterial DNA from Gram-positive Bacteria addition of Lysozyme is needed. Please add 5 µl of a 10 mg / ml Lysozyme-solution per 200 µl sample volume to the primary tube before starting the assay, incubate at 37°C or at RT for an adequate time before starting the assay.

Urine: The bacteria should be pelleted while the supernatant is discarded (urea contaminations can inhibit PCR reactions). For some applications fresh urine can be used directly. Best results are obtained with freshly pelleted material.

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

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 winning of the cell culture supernatant.

Invitak 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.

Preparation of starting materials

Attention: If your samples need Lysozyme, please incubate them outside the machine at RT or at 37°C

1. Extraction of NA from blood, serum, plasma, cell free body fluids, urine, liquor, transport media

This type of sample can be processed directly without any pre-preparations.

Please keep in mind that the first step in the equipment is premixing of samples. Samples have to be at least "pipetable", meaning the presence of clumps and other solid materials leads to clots and prevents a normal workflow of the process. We recommend strictly controlling samples for coagulation by mixing several times overhead before usage on the instrument.

2. Extraction of NA from rinsed liquid from swab samples

a) the sample will also be used for cultivation

Cut off the relevant part of the swab and transfer it into an RNase/DNase-free 2 ml tube. Add 400 µl physiological saline solutions to the swab and vortex intensely for 2-3 min and incubate for 10 min at RT. Take an aliquot for cultivation. Transfer 350 µl of the rinsed liquid into a primary tube.

optional: If bacterial DNA is processed, 20 µl Lysozyme can be added to 200 µl sample in 2.0 ml Collection Plate by the robot.

Note: This does not include any warranty for efficiency of the used cultivation method

b) the sample will not be used for cultivation

Cut off the relevant part of the swab and transfer this part into an RNase- and DNase-free 2 ml tube. Add 400 µl RNase-free water to the swab and vortex intensely for 3 min. Optional, incubate for 3 min at 95°C. Transfer 350 µl of the rinsed liquid into a primary tube

optional: If bacterial DNA is processed, 20 µl Lysozyme can be added to 200 µl sample in 2.0 ml Collection Plate by the robot.

3. Extraction of NA from samples like sputum, slimy tracheal secretes or BAL

a) Non-viscous samples

For isolation of bacterial DNA, transfer 1 ml of tracheal secret or BAL into a RNase/DNase-free tube and centrifuge at 11.100 x g (11.000 rpm) for 3 min. Discard / decant the supernatant without disturbing the bacterial pellet: Resuspend the bacterial pellet in 400 µl distilled water(1) or RNase free water and transfer the sample into a primary tube.

optional: If bacterial DNA is processed, 20 µl Lysozyme (not provided, stock solution 10 mg/ml) can be added to 200 µl sample in 2.0 ml Collection Plate by the robot.

For isolation of viral NA transfer 400 µl of the origin sample into a primary tube.

b) Viscous sample

Transfer 1 ml from the viscous sample into a RNase/DNase-free tube and add 1 ml saturated Acetylcysteine (ACC) solution to the sample (ratio sample to buffer must be 1:1).

Incubate the mixture for 10 min at 95°C under shaking on a thermomixer to reduce the viscosity.

For bacterial DNA extraction, centrifuge at 11.100 x g for 3 min. Discard the supernatant without disturbing the bacterial pellet. Resuspend the bacterial pellet in 400 µl distilled water or RNase-free water and transfer it into a primary tube.

optional: If bacterial DNA is processed, 20 µl Lysozyme (not provided, stock solution 10 mg/ml) can be added to 200 µl sample in 2.0 ml Collection Plate by the robot.

For viral NA extractions use directly 400 µl of the pretreated sample and transfer it into a primary tube. If the sample contains remaining solid particles, please avoid these by pipetting.

4. Extraction of viral NA from supernatant of stool suspension

Transfer 100 µl stool sample into a 2 ml tube and add 900 µl RNase-free Water. Vortex the sample for 30 s followed by a 1 min centrifugation step at 12.000 x g (13.000 rpm).

Transfer 400 µl virus containing supernatant into a primary tube (prevent the aspiration of swimming particles).

5. Extraction of bacterial NA from supernatant of stool suspension

Transfer 100 µl stool sample into a 2 ml tube and add 300 µl RNase-free Water. Vortex the sample for 30 s followed by a 30 s centrifugation step at 3.000 rpm. (1.000 x g)

Transfer 350 µl of the bacteria containing supernatant into the cavity of a primary tube (prevent the aspiration of swimming particles).

optional: If bacterial DNA is processed, 20 µl Lysozyme can be added to 200 µl sample in 2.0 ml Collection Plate by the robot.

Preparing of the internal control for the Hamilton®-system

Please read the instructions carefully and conduct the prepared procedure.

Using an internal control (IC)

Using the STARlet and the **InviMag® Universal Kit/ STARlet** in combination with commercially available amplification systems may require introducing an internal control (IC) into the purification procedure to monitor the efficiency of sample preparation.

Internal control DNA or RNA (IC) must be combined with the provided Carrier RNA solution of the **InviMag® Universal Kit/ STARlet** in one mixture. For each sample the machine transfers a volume of 10 µl of the stock solution to the lysis mix.

The vials with Carrier RNA solution of the **InviMag® Universal Kit/ STARlet** contain 1000 µl solution, so internal control for 100 samples must be added.

Example – Calculation:

Per Extraction 4.5 µl of an extraction control would be needed. It means per Sample that 10 µl of Carrier RNA stock solution must include 4.5 µl IC (= 45 %). The Rest up to 100 % (=55 %) will be RNase-free water. So fill the provided Carrier RNA tube with 550 µl RNase-free water (=55%) und 450 µl IC (=45%). Then mix it thoroughly.

Notes:

If you only have indication of amount per reaction, please calculate by using eluate and template volume.

If the internal control (IC) is stable in plasma, serum, CSF, urine, respiratory samples, whole blood, stool, transport media, or on dried swabs (e.g., armored RNA), it may be added to the sample shortly before the beginning of sample preparation. But consider that a bigger amount of internal control is necessary when using bigger volumes of primary sample tubes.

If the internal control (IC) is pure DNA or RNA, it is unstable in plasma, serum, CSF, urine, respiratory samples, whole blood, stool, transport media, or on dried swabs and should not be added directly to the samples.

Refer to the manufacturer's instructions to determine the optimal amount of internal control (IC) for specific downstream applications. Using an amount other than recommended may lead to wrong quantification results.

Preparing and loading of the Hamilton® system

Preparing the reagents:

Before starting with a new kit, add ethanol or isopropanol to the corresponding Wash-Buffers (Check tags on the bottles). Take an aliquot isopropanol from your stock-bottle into the provided Binding Solution Bottle (this avoids contamination of your stock-bottles)

Before starting a new run

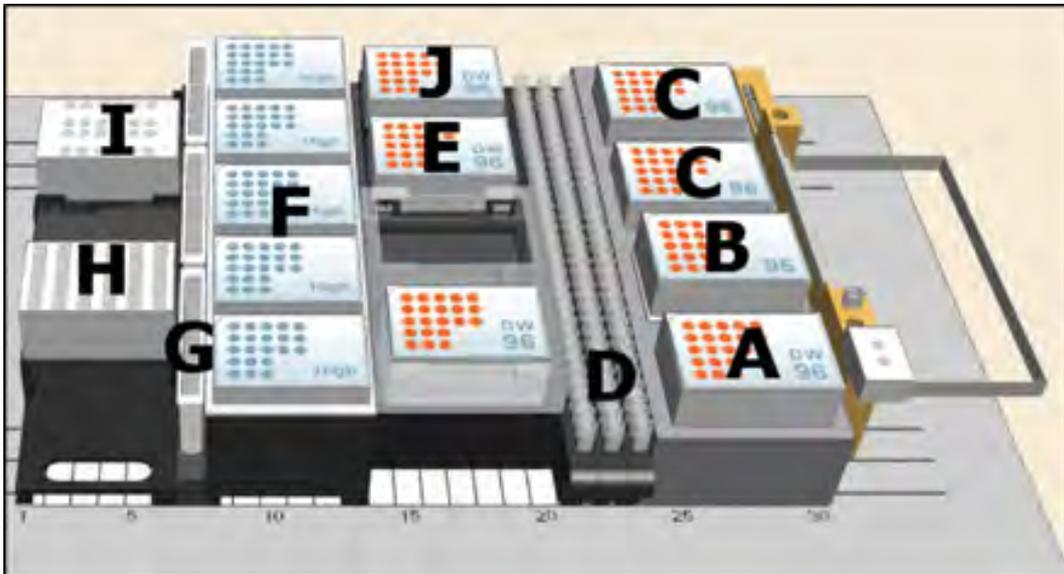
Dissolve (or unfreeze) four vials of Proteinase K and Carrier RNA. The four vials with SNAP Solution are ready to use. Each of the four vials is good for 192 samples.

Loading of the machine

The 96-well filter-plate can be used sequential. You may use any multiple of 8 wells on the plates in a first run and do the rest of the wells in later runs. This can be defined in the software but you have to seal the used wells with the provided sealing foils.

Do not forget to place a disposable bag in its holder.

Place one 96-deepwell plate on the heater-shaker (A) and two 2.0 ml Collection Plates on the waste positions (C).



Place the provided Elution Plate on Position E.



Please be advised that your STAR or STARlet platform may have a different deck layout and needs different positioning.

Load the samples into the machine and make sure you have enough liquid inside the sample tubes for a proper sample-transfer.

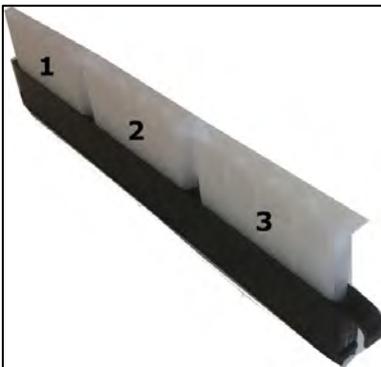
PLACE THE BLOOD SAMPLES IN FRONT OF ANY OTHER SAMPLE TYPE.

Load Lysis Buffer HLT, Elution Buffer M and Binding Solution into the smaller reagent trays referring to the sequences: Lysis Buffer in tray no.1; Elution Buffer in tray no. 2 and Binding Solution in tray no. 3.



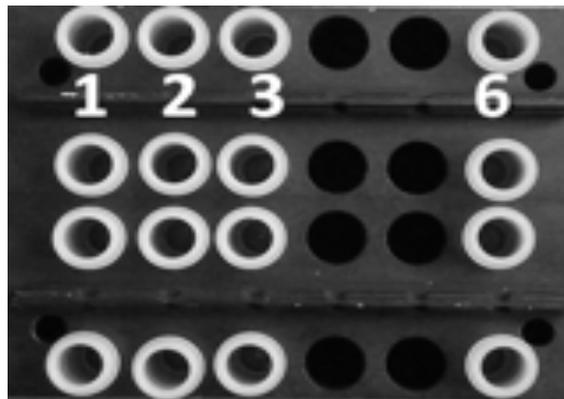
place the Tray Holder on position H

Load Wash Buffer HLT and Wash Buffer into the larger reagent trays referring to the sequences: Wash Buffer HLT in tray no: 1, Wash Buffer in tray No: 2 and 3.



place the Tray Holder on position G

Load Proteinase K in position **1** (4 tubes vertical), Carrier RNA in position **2** (4 tubes vertical) and SNAP Solution in position **3** (4 tubes vertical) of the reagent tray K. **In position 6 empty tubes (4 tubes vertical) are needed.** Please don't forget to place them in the 6th lane.



CHECK YOUR VENUS SOFTWARE FOR THE CORRECT SEQUENCE → DECK-POSITION

Invimag_Universal

This assay reuses the tips the most. Sample-transfer is done with the “reusable”-tips in the front of the tip-rack-holder. These tips are reused for the removal of the supernatant. For 96 samples you will need ~2.5 full racks of filter-tips.

Invimag_Universal_Blood

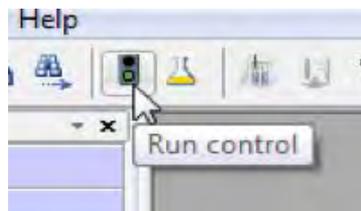
This assay is almost identical to the Invimag_Universal assay. The only difference is that the sample-transfer is done with fresh tips. Blood will clot the tip-head and render it unusable. For 96 samples you will need ~ 3.5 full racks of filter-tips.

If you are unsure how many tips will be used → make a test-run with a simulated machine.

When you are unsure if your sample will dry and clot inside the filter tip choose the Invimag_Universal_Blood assay.

Starting the machine

Startup the software and load the **Invimag_Universal** or **Invimag_Universal_Blood** assay. Switch to the Run Control of the software by clicking the small “stoplights” in the editor part.



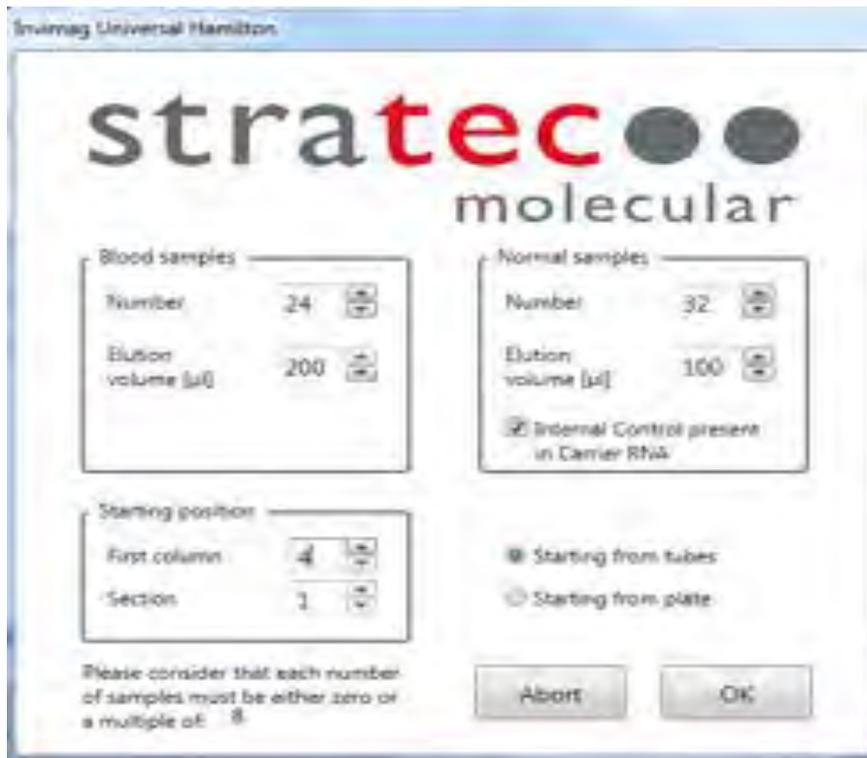
The Run Control of the Hamilton Software will open and check for connection to the STARLet machine.

Press “F5” or on the “Play Button” in the Run Control to start the assay.



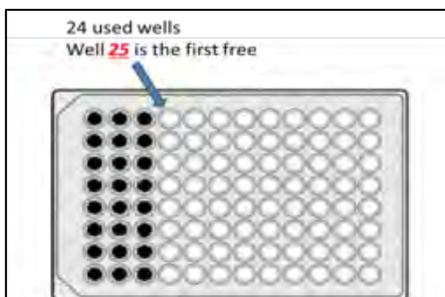
The machine will initialize and start a dialog prompting how many samples will be processed. It is necessary to use a multiple of 8 samples. In case of different numbers of samples, please complement to 8 with water samples.

Additionally, the instrument will ask for: the number of blood or normal-samples, for the first free lane in the Collection Plate, If the run should start from sample tubes or directly from a plate



Here, 24 blood samples and 32 normal samples are selected. Blood samples will be eluted in 200 µl, normal samples in 100 µl Elution Buffer M. The run will start from the 4th lane of the plates, starting from sample tubes.

used lanes - lane 4 is the first free lane:



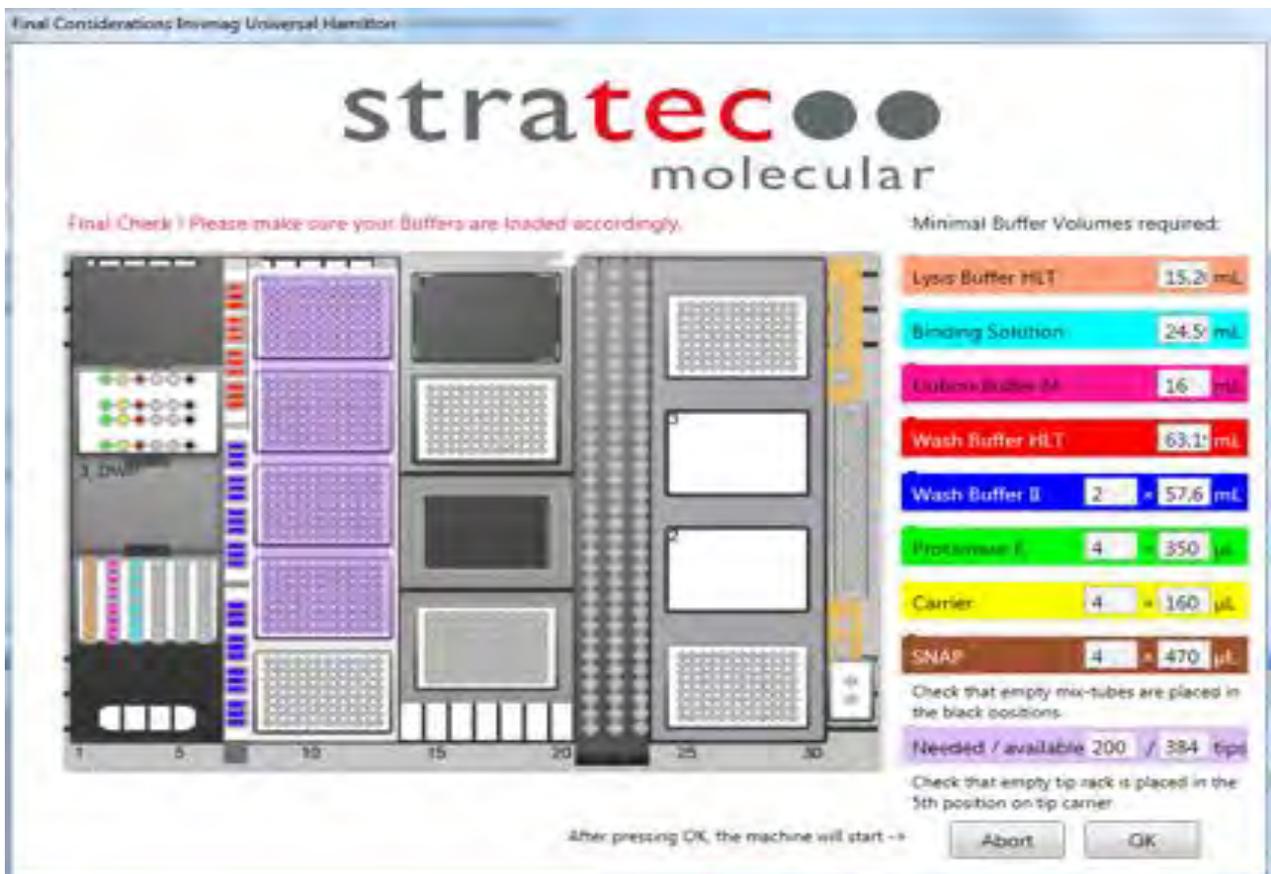
By pressing OKAY you get to the tip-definition screen. Please enter the amount of tips you have loaded into the machine.

Attention: The InviMag Assay needs one empty tip-rack to “park” the used tips for later usage.



Please ensure that the loaded filter-tips are shown at the right position of the picture. If not, this has to be adapted/ modified on the computer.

Pressing the button “OK” will switch to the next step.



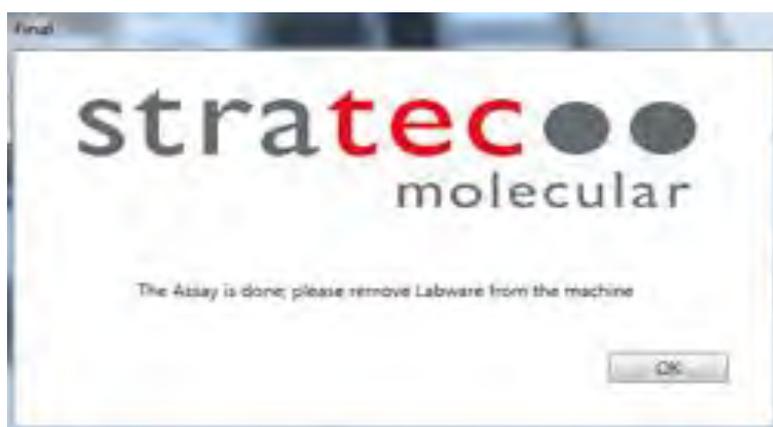
In this picture all buffers and amounts are displayed that are required for the number of selected samples. Fill the containers with the required buffer volumes and check that the buffers are located at the exact corresponding position.

Please make sure not to “overfill” the reagent trays as the Hamilton STARlet may misinterpret the detected Liquid Level and return an error message.

After pressing the button “OK”, the assay will be performed without any user-interference.

Please make sure the assay is performing unstopped as longer wait steps during lysis and washing steps may lead to less optimal DNA.

After run



Please seal the used wells of the Waste-plates and the Collection-plate with the provided Sealing Foils. Transfer the eluates from the Elution Plate to a suitable storage solution and seal the used wells with Sealing Foil as well.

Freeze the remaining Carrier RNA and Proteinase K. Store remaining SNAP Solution in a refrigerator.

Appendix

General notes on handling DNA/ RNA

Nature of DNA/ RNA

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

Handling fresh and stored material before the extraction of DNA/ RNA

For the isolation of DNA/ RNA use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at -70°C . This procedure minimizes degradation of crude DNA/ RNA by limiting the activity of endogenous nucleases.

Storage of DNA/ RNA

Store DNA/ RNA at $2-8^{\circ}\text{C}$. Storing DNA/ RNA at -20°C can cause shearing of DNA/ RNA, particularly if the DNA/ RNA is exposed to repeated freeze-thaw cycles. RNA should be stored for a long term storage at -80°C .

Troubleshooting

Problem	Probable cause	Comments and suggestions
Low amount of extracted DNA	Insufficient lysis	Reduce amount of starting material by diluting the samples beforehand
	Low amount of SNAP Solution	Mix SNAP Solution vigorously before use.
Low concentration of extracted DNA	Too much Elution Buffer	Elute the DNA with in a lower volume of Elution Buffer R . Change the volume in the run file to 50 µl.
	Incorrect storage of starting material	Ensure that the storage of starting material was correct. Avoid repeated thawing and freezing cycles of the sample material
	Incorrect Wash Buffers	Ensure, that the correct amount of ethanol is added to the Wash Buffers and stored correctly
Degraded DNA	Incorrect storage of starting material	Ensure that the storage of starting material was correct
	Old material	Ensure that the starting material is stored at appropriate conditions (-20°C/-80°C) avoid multiple thawing and freezing cycles of the material
DNA does not perform well in downstream-applications (e.g. real-time PCR or PCR)	No PCR result for genomic DNA	Some PCR-assays may not work in the presence of Carrier-RNA . Please use RNase-free water instead of the provided Carrier RNA . Due to the very gentle isolation procedure it may happen that isolated genomic DNA forms a cluster. To overcome this, the primary PCR denaturation step at 95°C should be prolonged to 5 min
	Ethanol carryover during elution	Ensure, that the correct amount of ethanol is added to the Wash Buffers and stored correctly Additionally check the position of the Deep-Well Plate on the magnetic position.
	Salt carry-over during elution	Check the Wash Buffers for salt precipitates. If there are any precipitates visible, solve them by carefully warming up to 30°C Ensure that the Wash Buffers are equilibrated at room temperature
Eluted DNA is brownish colored	Small part of the magnetic particles are left in the elution	Centrifuge at full speed for 1 min and transfer supernatant to a new tube

Problem	Probable cause	Comments and suggestions
Low amount of extracted viral RNA	No Carrier RNA added Low amount of SNAP Solution	Make sure enough Carrier RNA is added into the Deep-Well plate Mix SNAP Solution thoroughly before pipetting to the Deep Well Plate
Low concentration of extracted RNA	Too much Elution Buffer R Incorrect storage of starting material Incorrect Wash Buffers	Elute the DNA with in a lower volume of Elution Buffer R . Change the volume in the run file to 50µl. Ensure that the storage of starting material was correctly avoid repeated thawing and freezing cycles of the sample material Ensure that the correct amount of ethanol is added to the Wash Buffers
Degraded RNA	Incorrect storage of starting material Old material	ensure that the storage of starting material was correctly Avoid multiple thawing and freezing of the sample material Ensure that the starting material is fresh or stored at appropriate conditions (-20°C / -80 C) Avoid multiple thawing and freezing of the material
RNA does not perform well in downstream-applications (e.g. real-time RT-PCR or RT-PCR)	Ethanol carryover during elution Salt carryover during elution	increase drying time for removing of ethanol in the assay file 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 brownish colored	Small parts of the magnetic particles are left in the elution	Centrifuge at full speed for 1 min and transfer supernatant into a new tube

Ordering information

Product	Package Size	Catalogue No.
InviMag® Universal Kit / STARlet	24 x 96 preparations	7450330400
Related Products		
InviMag® Universal Kit/ IG	8 x 12 preps	2450120100
InviMag® Universal Kit /KF96	5 x 96 preparations	7450300300
Invisorb® Spin Universal Kit Kit	50 preparations	1050100200
Invisorb® Spin Universal Kit Kit	250 preparations	1050100300
InviMag® Pathogen Kit /KF96	5 x 96 preparations	7450300200
RTP® Pathogen Kit	50 preparations	1040500200
RTP® Pathogen Kit	250 preparations	1040500300

Possible suppliers for Isopropanol:

Carl Roth

2-Propanol
Rotipurán >99.7%, p.a., ACS, ISO
Order No. 6752

Applichem

2-Propanol
Order No. A3928

Sigma

2-Propanol
Order No. 59304-1L-F

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