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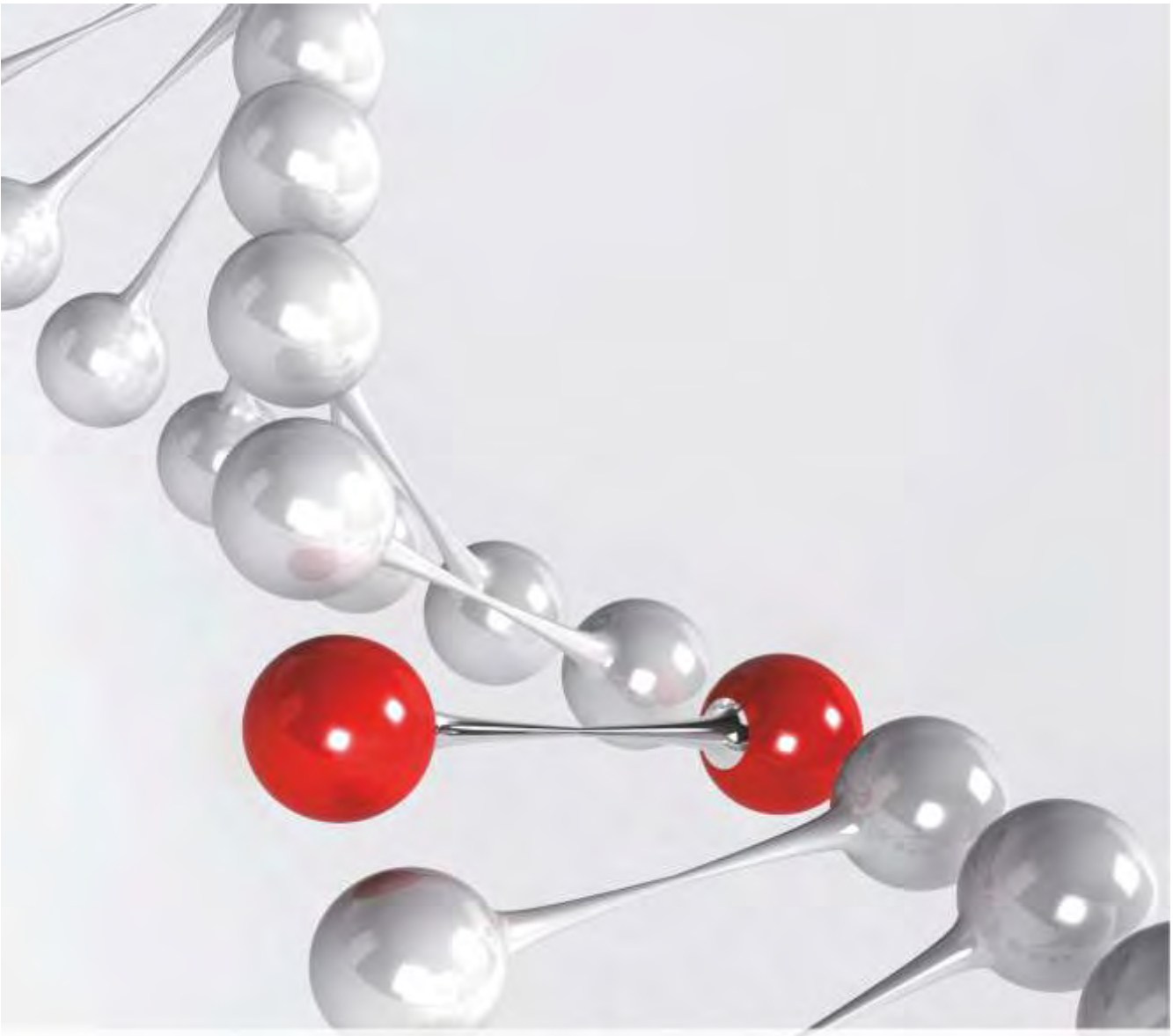

# User manual
# Invisorb® Universal HTS 96 Kit/ STARlet

## for use on the STARlet platform from Hamilton®

for walk away automated isolation and purification of total (genomic, bacterial) DNA and/or viral DNA/ RNA from up to 200 µl sample volume.

REF 7150330x00

STRATEC Molecular GmbH, D-13125 Berlin

# Instruction for Invisorb® Universal HTS 96 Kit/ STARlet

The **Invisorb® Universal HTS 96 Kit/ STARlet** combines the advantages of the innovative Invisorb® technology in combination with the STARlet platform from Hamilton® 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 **Invisorb® Universal HTS 96 Kit/ STARlet** was intensely tested and validated.

Due to the high purity of the 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.

**For research use only!**

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# Kit contents of Invisorb® Universal HTS 96 Kit/ STARlet

***Note:*** **2 ml Sarstedt Screw Tubes are necessary for preparation!**

| | 4 x 96 extractions | 24 x 96 extractions |
|---|---|---|
| **Catalogue No.** | 7150330300 | 7150330400 |
| **Lysis Buffer HLT** | 120 ml | 6 x 120 ml |
| **Proteinase K** | 8 | 48 |
| **Carrier RNA** | 8 | 48 |
| **RNase free Water** | 2 x 15 ml | 3 x 60 ml |
| **Binding Solution** | empty bottle (final volume 200 ml) | empty bottle (final volume 200 ml) |
| **Wash Buffer HLT** | 3 x 90 ml (final volume 3 x 150 ml) | 4 x 600 ml (final volume 4 x 1000 ml) |
| **Wash Buffer II** | 5 x 60 ml (final volume 5 x 200 ml) | 7 x 300 ml (final volume 7 x 1000 ml) |
| **Elution Buffer M** | 120 ml | 6 x 120 ml |
| **2.0 ml Collection Plate** | 4 | 24 |
| **Elution Plate L** | 4 | 24 |
| **DNA Binding Plate B** | 4 | 6 x 4 |
| **Sealing Foils** | 8 | 48 |
| **Initial steps** | Dilute each tube **Proteinase K** by addition of 1300 µl RNase free Water, mix thoroughly<br>Dilute each tube **Carrier RNA** by addition of 1000 µl RNase free Water, mix thoroughly<br>Fill 200 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle<br>Add 60 ml 99.7% Isopropanol to each bottle **Wash Buffer HLT.**<br>Add 140 ml of 96-100% ethanol to each bottle **Wash Buffer II.** Mix thoroughly and always keep the bottles firmly closed! | Dilute each tube **Proteinase K** by addition of 1300 µl RNase free Water, mix thoroughly<br>Dilute each tube **Carrier RNA** by addition of 1000 µl RNase free Water, mix thoroughly<br>Fill 200 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle<br>Add 400 ml 99.7% Isopropanol to each bottle **Wash Buffer HLT.**<br>Add 700 ml of 96-100% ethanol to each bottle **Wash Buffer II.** 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 **Invisorb® Universal HTS 96 Kit/ STARlet**, except **dissolved Carrier RNA** and **dissolved Proteinase K** should be stored at room temperature and are stable for at least 12 months.

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

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

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

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

**Wash Buffer** 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

STRATEC Molecular guarantees the correct function of the **Invisorb® Universal HTS 96 Kit/ STARlet** for applications as described in the manual. In accordance with STRATEC Molecular's certified QM-System each component of the **Invisorb® Universal HTS 96 Kit/ STARlet** was tested against predetermined specifications to ensure consistent product quality.

All products sold by STRATEC Molecular are subjected to extensive quality control procedures according to EN ISO 9001 and EN ISO 13485 and are warranted to perform as described when used correctly. Any problems should be reported immediately.

STRATEC Molecular reserves the right to change, alter or modify any product to enhance its performance and design at any time.

If you have any questions or problems regarding any aspects of **Invisorb® Universal HTS 96 Kit/ STARlet** or other STRATEC Molecular products, please do not hesitate to contact us**.**

**For technical support or further information please contact:**

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

## Intended use

The **Invisorb® Universal HTS 96 Kit/ STARlet** has been designed for fully automated extraction and purification of viral DNA/ RNA, genomic DNA, bacterial DNA from 96 samples per run 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 **Invisorb® Universal HTS 96 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* (only 100µl), 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 **Invisorb®** technology, which relies on binding of the nucleic acids on silica surfaces. The procedure only requires minimal user interaction (prefilling of the plates and containers), 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 INDENTED FOR USE BY PROFESSIONAL USERS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA/ RNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings. To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

## Product use limitation

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 STRATEC Molecular Product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the Product with respect to specific applications.

STRATEC Molecular products may be used e.g. in clinical diagnostic laboratory systems under following conditions:

- If used in the US, based on the condition that the complete diagnostic system of the laboratory has been validated pursuant to CLIA’ 88 regulations.
- For other countries based on the condition that the laboratory has been validated pursuant to equivalents according to the respective legal basis.

All Products sold by STRATEC Molecular are subject to extensive quality control procedures (according to EN ISO 9001 and EN ISO 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and 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.molecular.stratec.com** for each STRATEC Molecular Product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the waste generated by the **Invisorb® Universal HTS 96 Kit/ STARlet** procedures 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 **Invisorb® Universal HTS 96 Kit/ STARlet,** to which they apply, are listed are listed below as follows:

**Proteinase K**

Danger

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

**Lysis Buffer HLT**

Warning

H302-H315-H319-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

**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 Invisorb® Universal HTS 96 Kit/ STARlet

The **Invisorb® Universal HTS 96 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 with the STARlet, a Hamilton® robotic platform. The **Invisorb® Universal HTS 96 Kit/ STARlet** provides 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 |
|---|---|---|
| 100 µl fresh or frozen blood*,<br>200 µl fresh or frozen plasma/ serum<br>200 µl cell free body fluids (urine)<br>200 µl rinsed liquid from swab<br>200 µl transport media (Surepath, Thinprep)<br>200 µl supernatant from stool suspension<br>200 µl from liquid sputum, BAL, tracheal secrete<br><br>*blood, plasma: EDTA /Citrate stabilized, but no heparine | depending on sample (storage and source)<br>***Note:***<br>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 100 min |

The Starlet instrument uses an 8 channel pipettor to transfer samples and buffers and a vacuum station to separate the DNA/ RNA 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** (not provided) too if required), binding conditions are adjusted upon addition of **Binding Solution.** The genomic DNA and/or viral DNA/ RNA bind to DNA Binding Plate B and is separated from the solution by the vacuum pump controlled by the STARlet system. Subsequent to three washing steps of the bound nucleic acids, the nucleic acids are finally eluted in **Elution Buffer M**.

The instrument provides one run file for using the **Invisorb® Universal HTS 96 Kit/ STARlet** with the assay name: Invisorb_Universal_Starlet 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

For the isolation of DNA only from blood samples, STRATEC Molecular offers beside spin kits and blood kits for KingFisher™ family the InviMag® and Invisorb® Blood Mini Kit /STARlet for 8–96 samples, as well as the Invisorb® Blood Mini 96 HTS Kits for use on a centrifuge or robotic station.

For the isolation of viral RNA, DNA or both, STRATEC Molecular offers a series of spin kits as well as HTS kits for use on centrifuge, or for a walk-away automated isolation on robotic stations, as well as magnetic bead based kits for KingFisher™.

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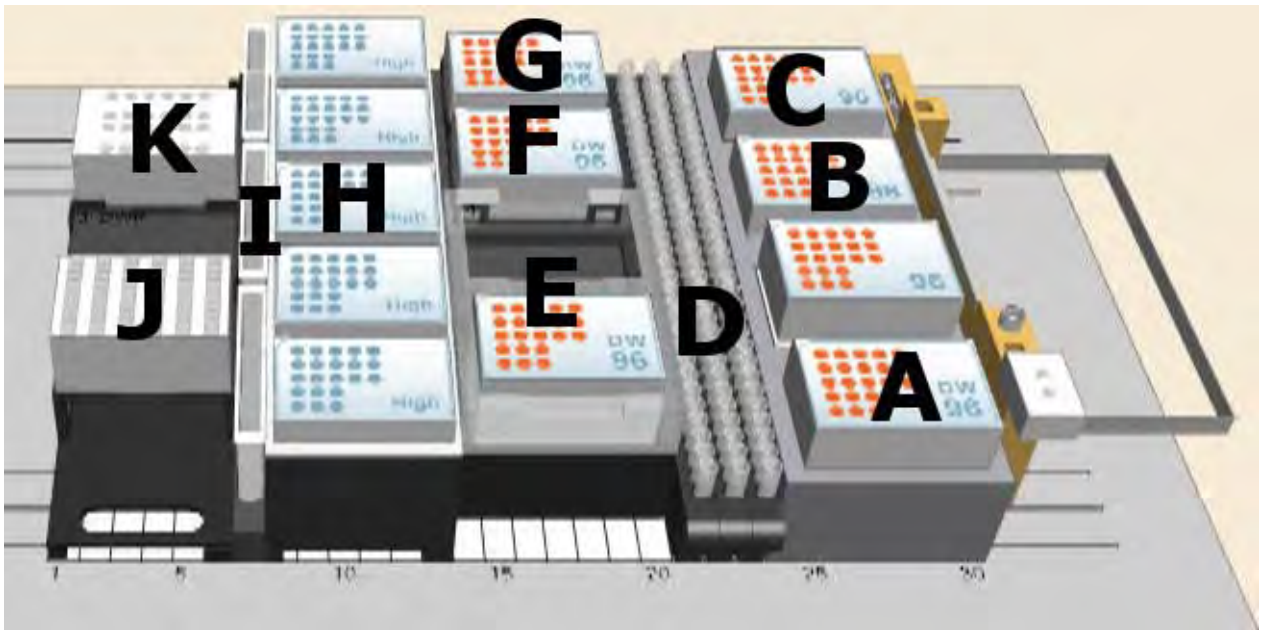
## Equipment characteristics of the STARlet platform

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 (H) as a barrier between the “clean” and “dirty” areas on the machine. Clean Reagent trays are placed on the left side (K, J 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), C (Parking Position for the Channeling Plate), F (Elution Plate L) comprise the 3 needed Deep Well Plate positions. Position E is the vacuum-station which includes the vacuum position and the filter plate holder.

Picture of the status before running the assay

Picture of the status at the end of the assay – elution plate on position E

## Principle and procedure

The **Invisorb® Universal HTS 96 Kit / STARlet** procedure comprises following steps:

- sample preparation if needed
- lysis and protein digestion
- binding of the DNA/ RNA on the membrane of the filter plate
- washing the membrane bound DNA/ RNA
- elimination of alcohol
- elution of DNA/ RNA.

After lysis the DNA/ RNA binds to the filter plate, 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 the 2.0 ml Collection 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** to the lysate, the DNA/ RNA are bound to the filter plate.

**Removing residual contaminants**
Contaminants are efficiently washed away while the DNA/ RNA remains bound to the filter plate.

**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 **Invisorb® Universal HTS 96 Kit/ STARlet** procedure from whole blood depends on the leucocytes content, the sample source, transport, storage, and age.
Typically, a volume of 100 µl of a whole blood sample from a healthy individual with a white blood cell content - ranging from $3x10^6$ to $1x10^7$ cells/ ml - will yield at least 1 µg of genomic DNA. The typical yield usually expected from the **Invisorb® Universal HTS 96 Kit/ STARlet** is about 1-3 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 **Invisorb® Universal HTS 96 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 from this kit contain genomic or pathogen DNA / RNA and Carrier RNA and amounts of Carrier RNA will greatly exceed amounts of viral nucleic acids.

Yields of viral NA isolated from biological samples are very low and therefore difficult to determine photometrically.* Keep in mind that the Carrier RNA (~5 µg per 200 µl sample) will account for most of the RNA presence.

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.

* In Gel Electrophoresis and in Capillary Electrophoresis, RNA extracted with the provided kit looks like degraded because 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, inspect the product and its components as well as the package for any apparent visible damages, correct quantities. If there are any unconformities please notify STRATEC Molecular. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 6). 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 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
- This kit should only be used by trained personnel.

# Preparing reagents and buffers

| **4 x 96 extractions:** |
|---|
| Dilute each tube **Proteinase K** by addition of 1300 µl RNase free Water, mix thoroughly and store like described above! |
| Dilute each tube Carrier RNA by addition of 1000 µl RNase free Water, mix thoroughly and store like described above! |
| Fill 200 ml >99.7% **Isopropanol** (molecular biologic grade) into the empty bottle. |
| Add 60 ml >99.7% Isopropanol to each bottle **Wash Buffer HLT.** |
| Add 140 ml of 96-100% ethanol to each bottle **Wash Buffer II.** Mix thoroughly and always keep the bottles firmly closed. |
| **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 200 ml >99.7% **Isopropanol** (molecular biologic grade) into the empty bottle. |
| Add 400 ml >99.7% Isopropanol to each bottle **Wash Buffer HLT.** |
| Add 700 ml of 96-100% ethanol to each bottle **Wash Buffer II.** Mix thoroughly and always keep the bottles firmly closed! |

## Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Conductive Tips with Filter, 1ml***
- Disposable gloves
- $ddH_2O$
- Vortex
- 96-100% ethanol
- 99.7% Isopropanol * (molecular biological grade)
- 2 ml micro tubes (Sarstedt: REF: 72.609, 2 ml PP)

*The **Invisorb® Universal HTS 96 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

*****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 **Invisorb® Universal HTS 96 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 3 plates
- Vacuum station for Hamilton Robotics
- Vacubrand Vacuum station (usually provided by Hamilton if desired)
- 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 12

# 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 (100µl blood).*

***STRATEC Molecular will not take responsibility if other kinds of samples are used than the sample types described or if the prepared procedures are modified.***

**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 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 because this may cause degradation**.** Various different primary tubes, blood collection system (e.g. Sarstedt, Greiner) and anticoagulants (except heparin) can be used to collect blood samples for the Invisorb® Universal HTS 96 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.

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 (not provided) 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 application 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.

STRATEC Molecular will be released of its responsibilities if other sample materials than described in the “Intended Use” are processed or if the sample preparation protocols are changed or modified.

## 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”, mean the presence of clumps and other solid materials may lead 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 will be processed 20 µl Lysozyme can be added to 200 µl sample in the 2 ml Collection Plate outside 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 will be processed 20 µl Lysozyme can be added to 200 µl sample in the 2 ml Collection Plate outside the robot.*

### 3. Extraction of NA from sputum

Transfer 200 µl from the sputum sample into an RNase/DNAse-free tube and add 200 µl NAC Buffer (order number: 1033221100) or saturated Acetylcysteine (ACC) solution to the sample (ratio sample to buffer must be 1:1). Incubate the mixture for 10 min at 95°C to reduce the viscosity and transfer 350 µl from the mixture into the primary tube.

*optional:* *If bacterial DNA will be processed 20 µl Lysozyme can be added to 200 µl sample in the 2 ml Collection Plate outside the robot.*

### 4. Extraction of NA from slimy tracheal secretes or BAL

**Non viscous samples:** Transfer 1 ml of tracheal secret or BAL into a RNase/DNAse-free tube and centrifuge at 9.300 x *g* (10.000 rpm) 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 the sample into a primary tube,

*optional:* *If bacterial DNA will be processed 20 µl Lysozyme can be added to 200 µl sample in the 2 ml Collection Plate outside the robot.*

**Viscous sample:** Transfer 1 ml of tracheal secrete or BAL into a RNase- and DNAse-free tube and add 1 ml NAC Buffer (order number: 1033221100) or saturated Acetylcysteine (ACC) solution to the sample (ratio sample to buffer must be 1:1)

Incubate the mixture for 10 min at 95°C to reduce the viscosity and centrifuge at 9.300 x *g* (10.000 rpm) for 3 min. Discard the supernatant without disturbing the bacterial pellet directly.

Resuspend the bacterial pellet in 400 µl distilled water or RNase free water and transfer it into a primary tube,

optional: *If bacterial DNA will be processed 20 µl Lysozyme can be added to 200 µl sample in the 2 ml Collection Plate outside the robot.*

### 5. 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).

### 6. 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 will be processed 20 µl Lysozyme can be added to 200 µl sample in the 2 ml Collection Plate outside 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 **Invisorb® Universal HTS 96 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 **Invisorb® Universal HTS 96 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 **Invisorb® Universal HTS 96 Kit/ STARlet** contain 1000 µl solution, so internal control for 100 samples must be added.

### Example – Calculation (IC):

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 you start 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) 4 vials of Proteinase K and Carrier RNA. These vials are good for 192 samples.

**Loading of the machine**

**The description is for the configuration in the following picture.**

## *Warning: Please be advised that your STAR or STARlet platform may has a different deck layout and needs different positioning.*

The 96-well filter-plate can be used sequential (in two parts). You may use any multiple of 8 wells on the plates in a first run and do the rest of the wells in a second run. Be sure to seal all wells which are not used in the current assay.

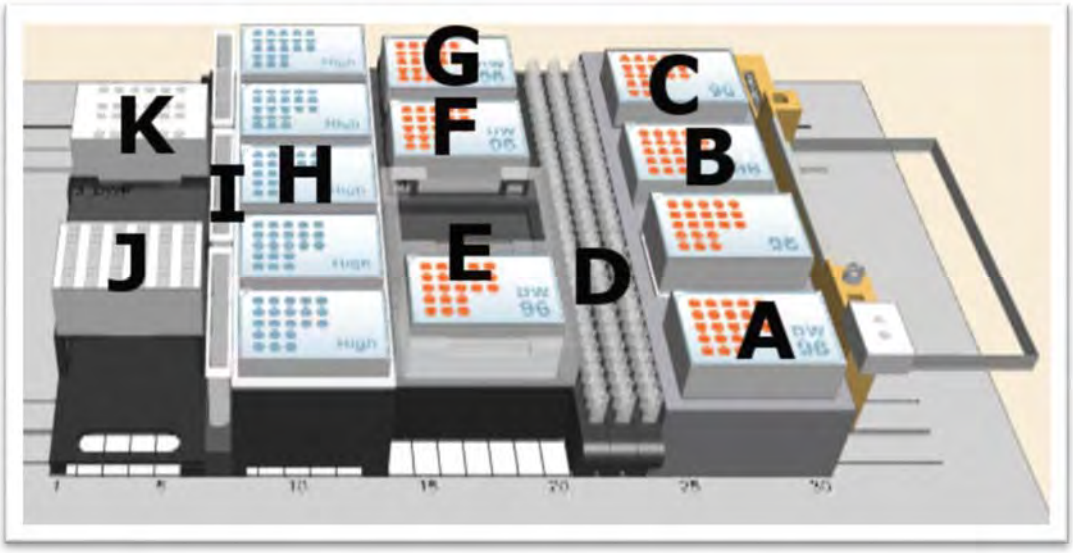
**It is very important for the vacuum steps to seal the filter-plate on the unused wells.**

Place one 96-deepwell plate on the heater-shaker (A) and one 96-elution plates on the position F. After the run the eluates will be located in the Elution Plate L on position E in the Vacuum Station.

Please seal the used wells of the Filter- and Collection-Plate with the provided Sealing Foil. Remaining free cavities of plates can then be used later without risks of contaminations.

For easier cleanup place a small piece of paper-tissue on position C as the channeling plate may be wet underneath.

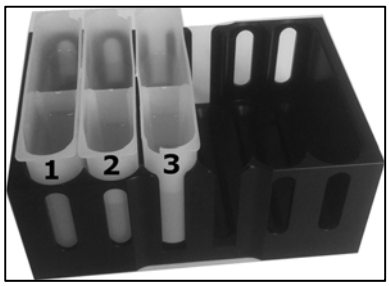
Don´t forget to place a disposable bag in its holder.




Load the samples into the machine and make sure that enough liquid is available inside the sample tubes for a proper sample-transfer. Please consider that different sample tubes have different dead volumes.

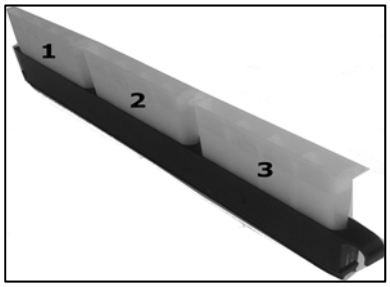
**PLACE THE BLOOD SAMPLES IN FRONT OF ANY OTHER SAMPLE TYPE.**

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.



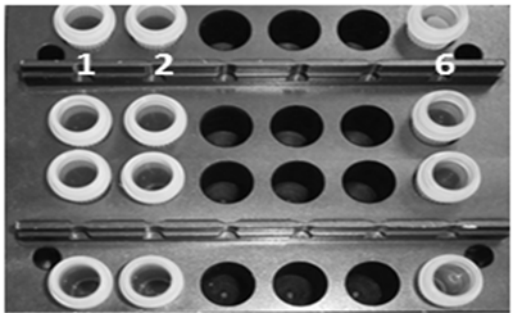

Place the Tray Holder on position J

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.

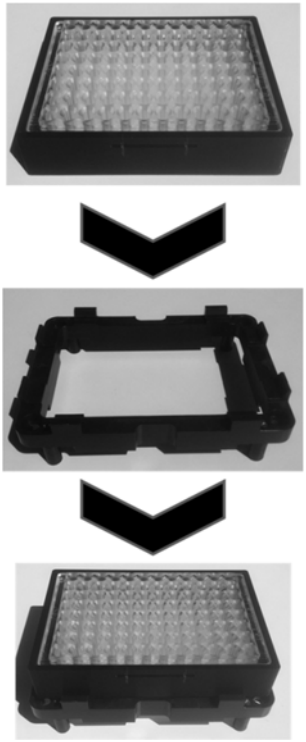



Place the Tray Holder on position I

Load Proteinase K in position **1** (4 tubes vertical) and Carrier RNA in position **2** (4 tubes vertical) of the reagent tray K. In position **6** empty tubes are needed.




**Preparation of the Vacuum Station**



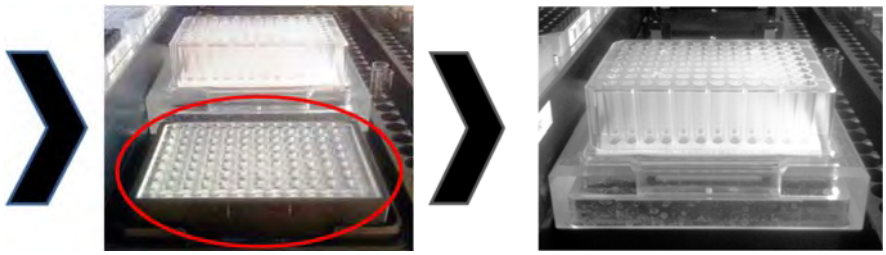
1) Place the transparent Channeling Plate on the black holder of the Hamilton Starlet platform. Make sure you cleaned and dried the Channeling Plate before doing a new run. The flat side has to be up.

2) Place the Channeling Plate inside the Hamilton Spacer for the Vacuum station

3) The complete construct is placed inside the Vacuum Station.

4) Finally the Filter-Plate with the transparent Filter-Plate-Holder is placed on top of the Vacuum station

5) Make sure you see the sealing gasket of the Vacuum station by pressing firmly on the Filter-Plate to ensure correct Vacuum during the filtration process.



Place the filter-tips on the corresponding tip-sequences. The Invisorb_Universal_Starlet assay employs non-reusable filter-tips

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

**Starting the machine**

Startup the software and load the Invisorb_Universal_Starlet Assay

Press on Run Control to start the machine.

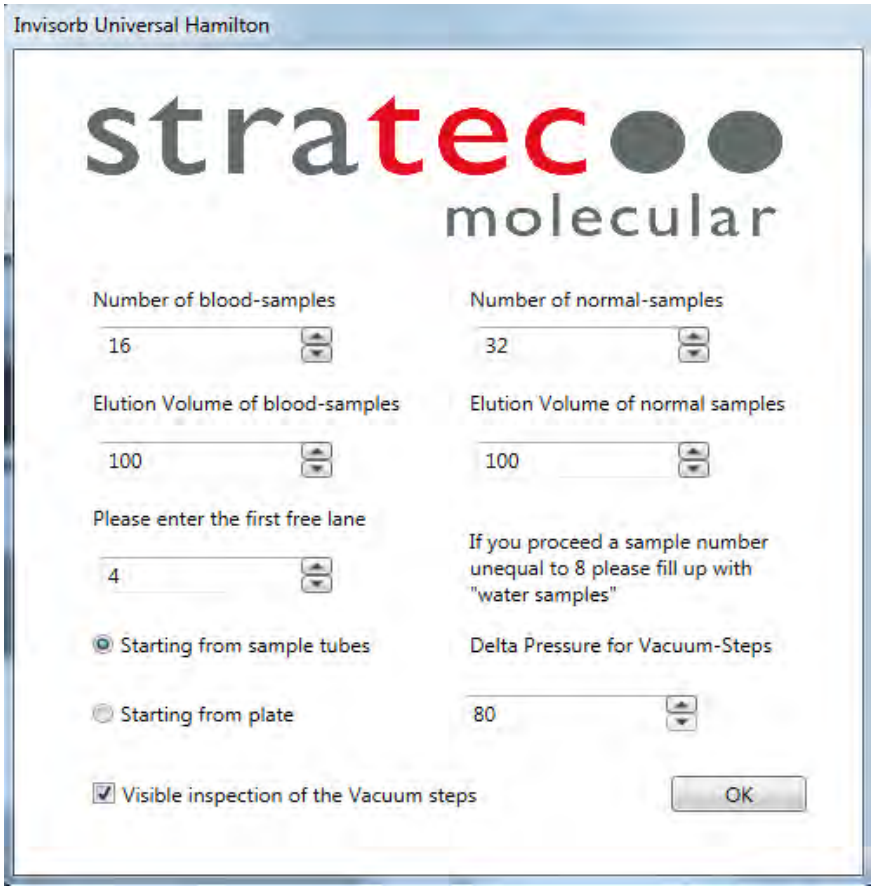
**Run control**

Change to the RUN-CONTROL of the Venus-Software and select START. 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 number of samples, please complement to 8 with water samples.

**Additionally, the instrument will ask for:**

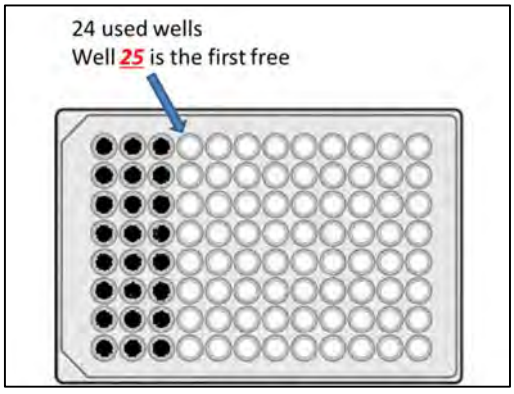
the number of blood or normal-samples,
for the first free lane in the Collection Plate,

If the run should start from sample tubes or directly from a plate
and if the filterplate should be inspected after the two first Vacuum steps.



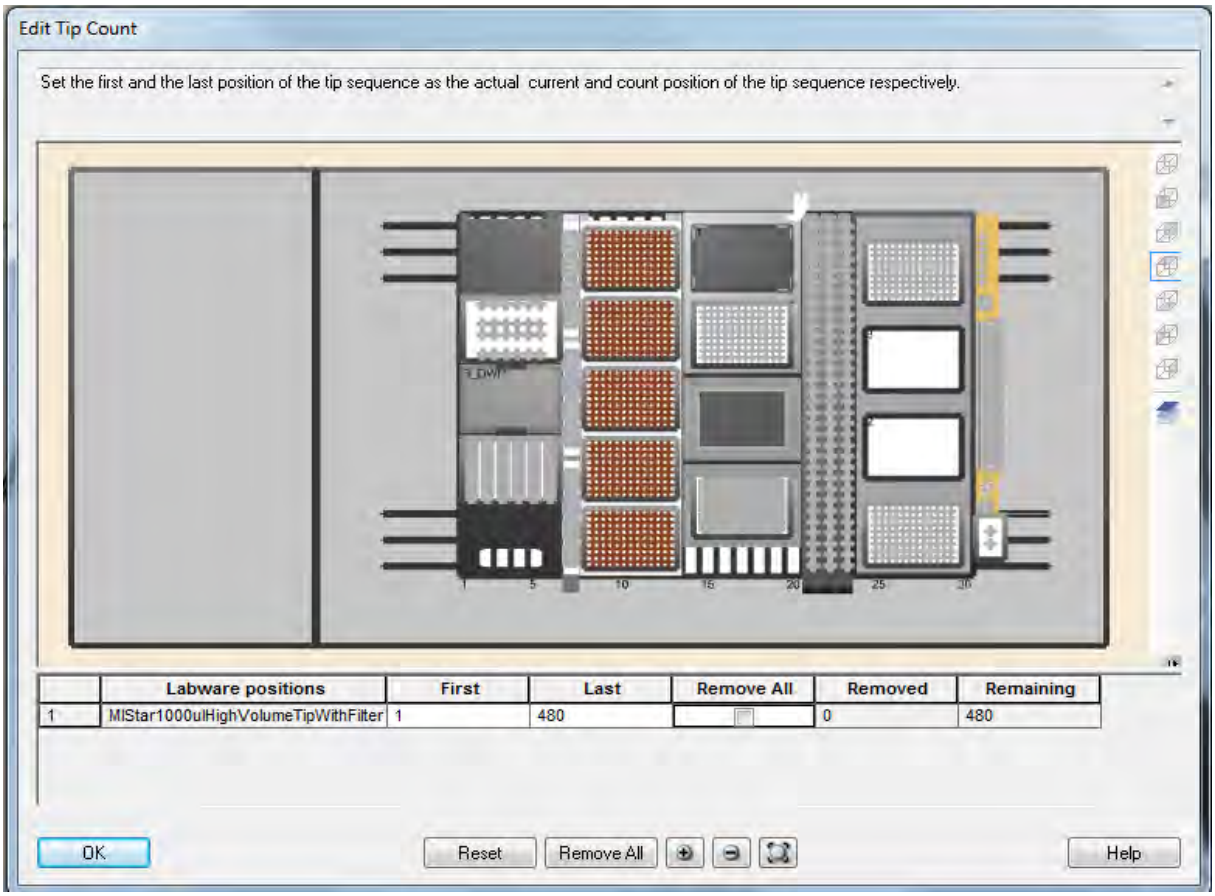

Here, 16 blood samples and 32 normal samples are shown. All samples will be eluted in 100 µl Elution Buffer M. The run will start from the 4th lane of the plate, starting from sample tubes and it will stop for visible inspection of the Filterplate ofter the vacuum steps.

3 used lanes - lane 4 is the first free lane:



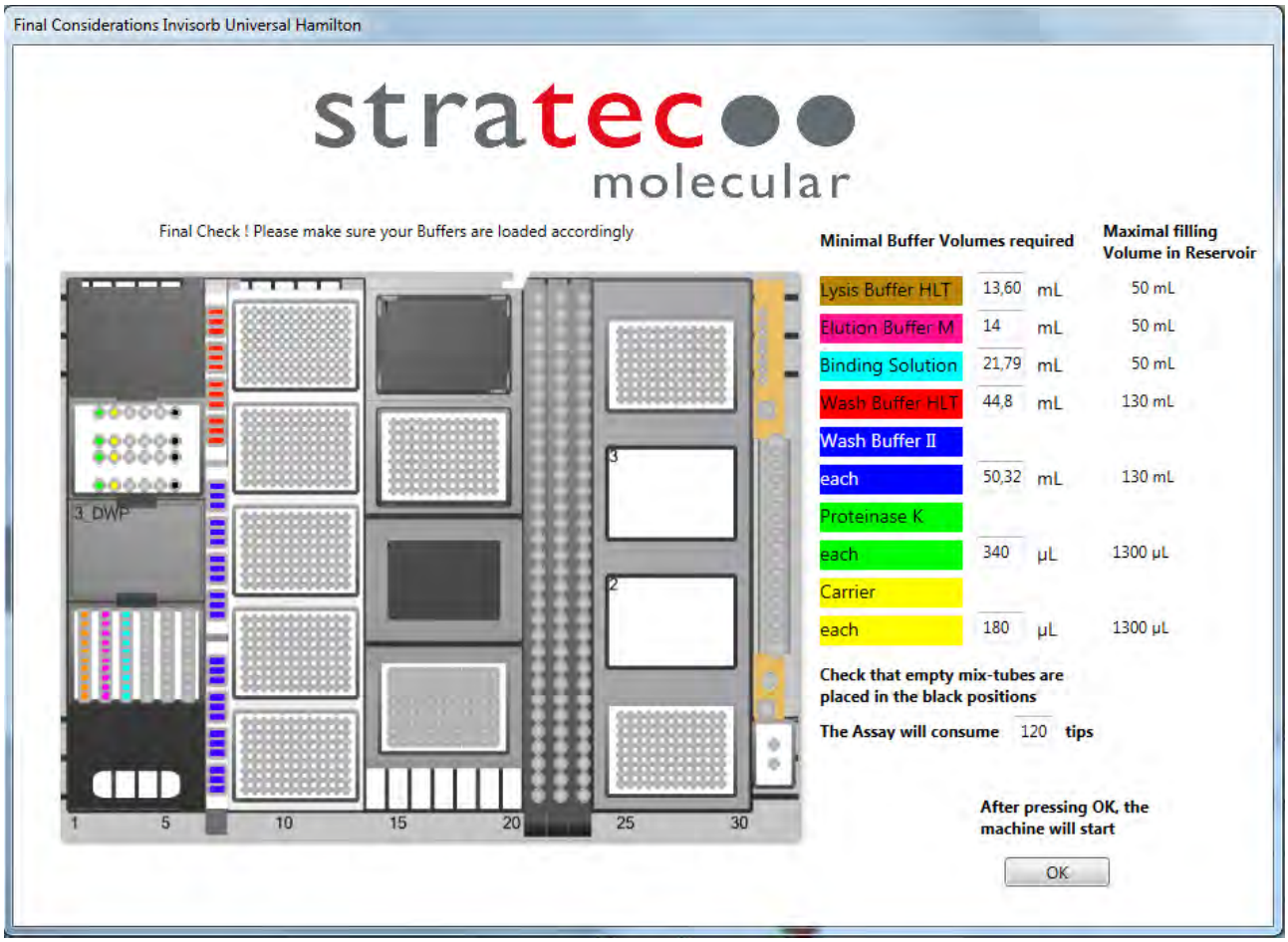

***If the button to inspect the Filterplate is pressed after the first two vacuum steps, ensure to watch the Hamilton Starlet or at least be present in the room to inspect the plate and to continue the assay. In case of too long waiting time, the assay performance will be decreased.***

After that, the required amount of filter-tips must be entered. Please assure to load enough tips for the run. Reloading tips during an assay may decrease the quality of the DNA because the machine will stop when tips are missing.




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.

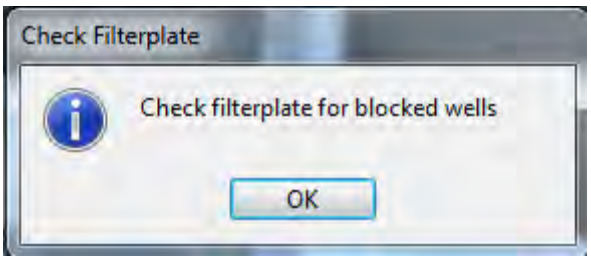
Pressing the button “OK” will switch to the next step.




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

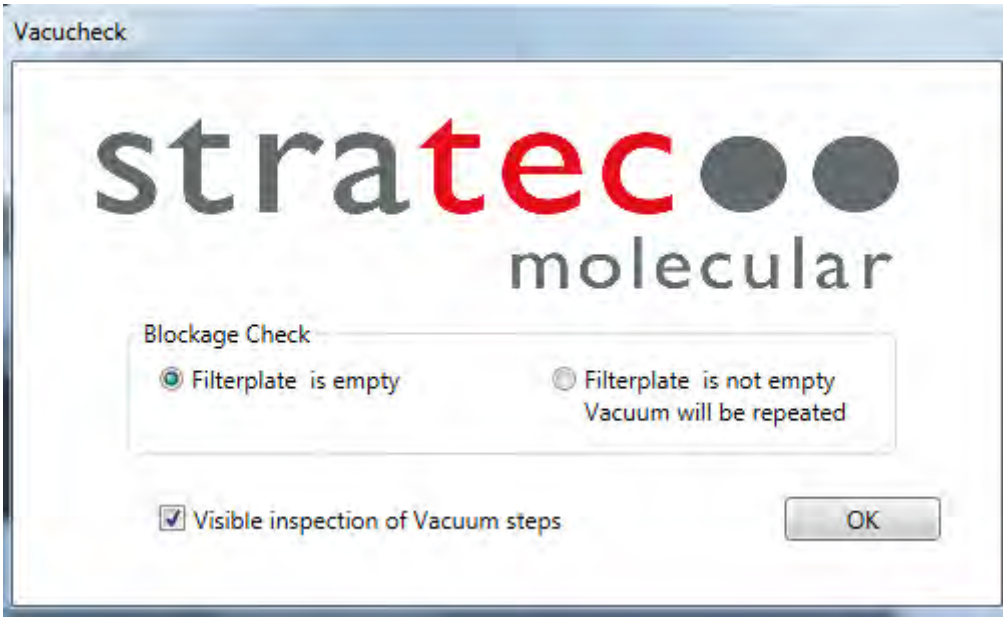
**After pressing the button “OK”, the assay will be performed without any user-interference if no visible inspection after the first two vacuum steps was selected.**

**If a visible inspection** should be performed, an alarm will be played after about 30 minutes. The required time can vary in dependence to the number and kind of samples. Please be present at the machine in order not to cause a too long break for inspection because this may lead to poor results.

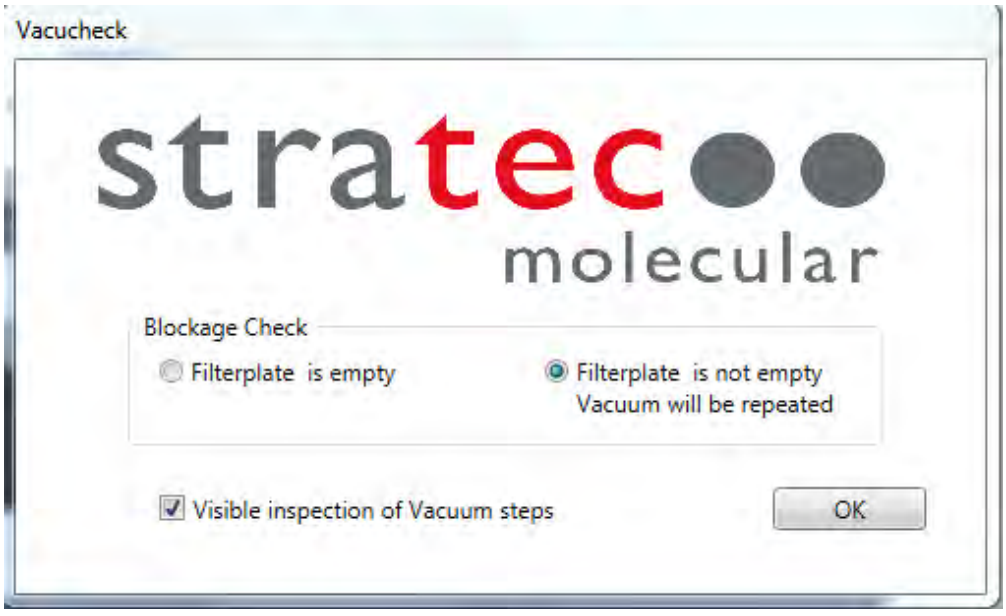



Pressing the button “OK” will end the alarm.

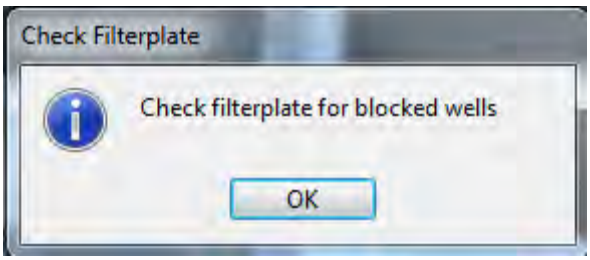
The machine is stopped. Inspecting the filter-plate for blocked wells is possible at this point.




If the field “Filterplate is empty” and “Visible inspection of Vacuum steps” is selected and the button “OK” is pressed, the assay will continue and asks again after the second vacuum step. If everything looks good, click the button “OK” again and do not select “Visible inspection of Vacuum steps”. The assay will continue until the end without any additional user interaction.

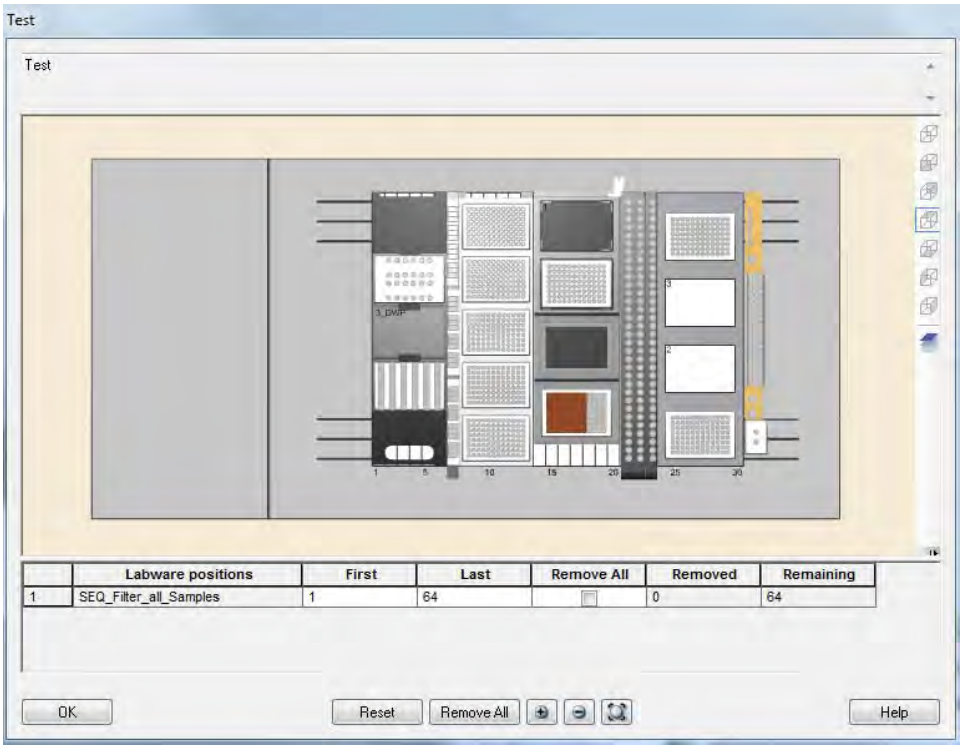



If the field “Filterplate is not empty” is selected, another vacuum step will be performed.



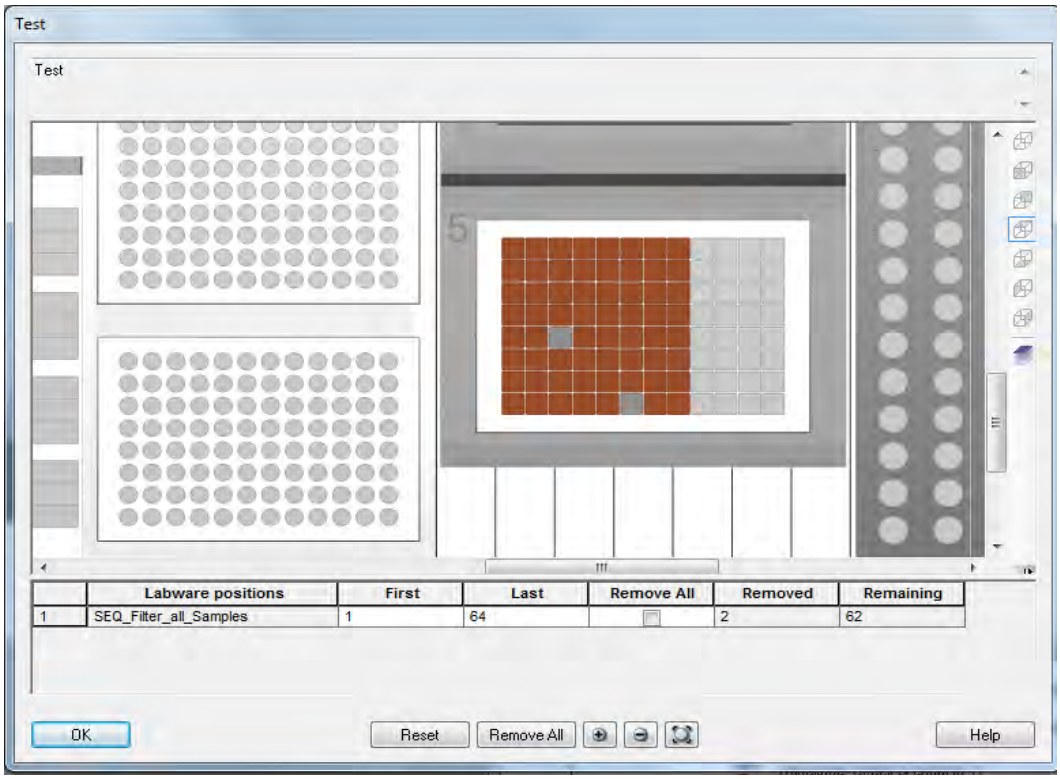

Pressing the button “OK” will end the alarm.

The machine is stopped. Inspecting the filter-plate for blocked wells is possible at this point.




Well E3 and H6 are blocked.

Mark the blocked wells on the computer like shown beneath and press the “OK” button. Those wells will be left open until the end of this run.




In this case the assay will continue and ask again after the next vacuum step. If everything looks good, press the button “OK”. The assay will run until the end without any further user interaction if you remove the check mark for visible inspection. If not it will ask again.

If there are more blocked wells the same pictures will appear and the blocked wells can be complemented. Go on like described above.

**After run**


Please seal the used wells of the Filterplate and the Collection plate with the provided Sealing Foils. Transfer the eluates from the Elution Plate to a suitable storage solution or seal the Elution Plate.

Freeze the remaining Carrier RNA and Proteinase K. Discard the remaining buffers and clean all used containers. If required, decontaminate the machine with UV-light. Please take the plate-holder out of the vacuum-station before applying UV-light and clean it using (fresh) tap water.

The rubber seal of the plate-holder for the vacuum-station needs to be groomed with oil or glycerol from time to time.

# 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°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. Virus 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 concentration of extracted DNA** | Too much Elution Buffer | Elute the DNA with in a lower volume of **Elution Buffer M**. Change the volume in the run file to 50 µl. |
| | Incorrect storage of starting material | Ensure that the storage of starting material was correct.<br>Avoid repeated thawing and freezing cycles of the sample material |
| | Incorrect Wash Buffers | Ensure, that the correct amount of ethanol/ isopropanol 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)<br>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 Buffer** and stored correctly |
| | 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<br>Ensure that the **Wash Buffers** are equilibrated at room temperature |

| Problem | Probable cause | Comments and suggestions |
|---|---|---|
| **Low amount of extracted viral RNA** | No Carrier RNA added | Make sure enough **Carrier RNA** is added into the Deep-Well plate |
| **Low concentration of extracted RNA** | Too much Elution Buffer M | Elute the RNA with in a lower volume of **Elution Buffer R**. Change the volume in the run file to 50µl. |
| | Incorrect storage of starting material | Ensure that the storage of starting material was correctly<br>avoid repeated thawing and freezing cycles of the sample material |
| | Incorrect Wash Buffers | Ensure that the correct amount of ethanol/ isopropanol is added to the **Wash Buffers** |
| **Degraded RNA** | Incorrect storage of starting material | ensure that the storage of starting material was correctly<br>Avoid multiple thawing and freezing of the sample material |
| | Old material | Ensure that the starting material is fresh or stored at appropriate conditions (-20°C / -80 C)<br>Avoid multiple thawing and freezing of the material |
| **RNA does not perform well in downstream-applications (e.g. real-time RT-PCR or RT-PCR)** | Ethanol carryover during elution | increase drying time for removing of ethanol in the assay file |
| | Salt carryover during elution | Check the **Wash Buffers** for salt precipitates. If there are any precipitates visible, solve them by carefully warming up to 30°C<br>Ensure that the **Wash Buffers** are equilibrated at room temperature |

# Ordering information

| Product | Package Size | Catalogue No. |
|---|---|---|
| Invisorb® Universal HTS 96 Kit / STARlet | 4 x 96 preparations | 7150330300 |
| Invisorb® Universal HTS 96 Kit / STARlet | 24 x 96 preparations | 7150330400 |

| Related Products | Package Size | Catalogue No. |
|---|---|---|
| InviMag® Universal Kit / STARlet | 4 x 96 preparations | 7450330300 |
| InviMag® Universal Kit / STARlet | 24 x 96 preparations | 7450330400 |
| InviMag® Universal Kit/ IG | 8 x 12 preparations | 2450120100 |
| InviMag® Universal HTS 96 Kit /KF96 | 1 x 96 preparations | 7450300200 |
| InviMag® Universal HTS 96 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 | 1 x 96 preparations | 7450300100 |
| InviMag® Pathogen Kit /KF96 | 5 x 96 preparations | 7450300200 |
| InviMag® Pathogen Kit/ KFmL | 15 preparations | 2450110200 |
| InviMag® Pathogen Kit/ KFmL | 75 preparations | 2450110300 |
| RTP® Pathogen Kit | 50 preparations | 1040500200 |
| RTP® Pathogen Kit | 250 preparations | 1040500300 |

### Possible suppliers for Isopropanol:

**Carl Roth**
2-Propanol
Rotipuran >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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