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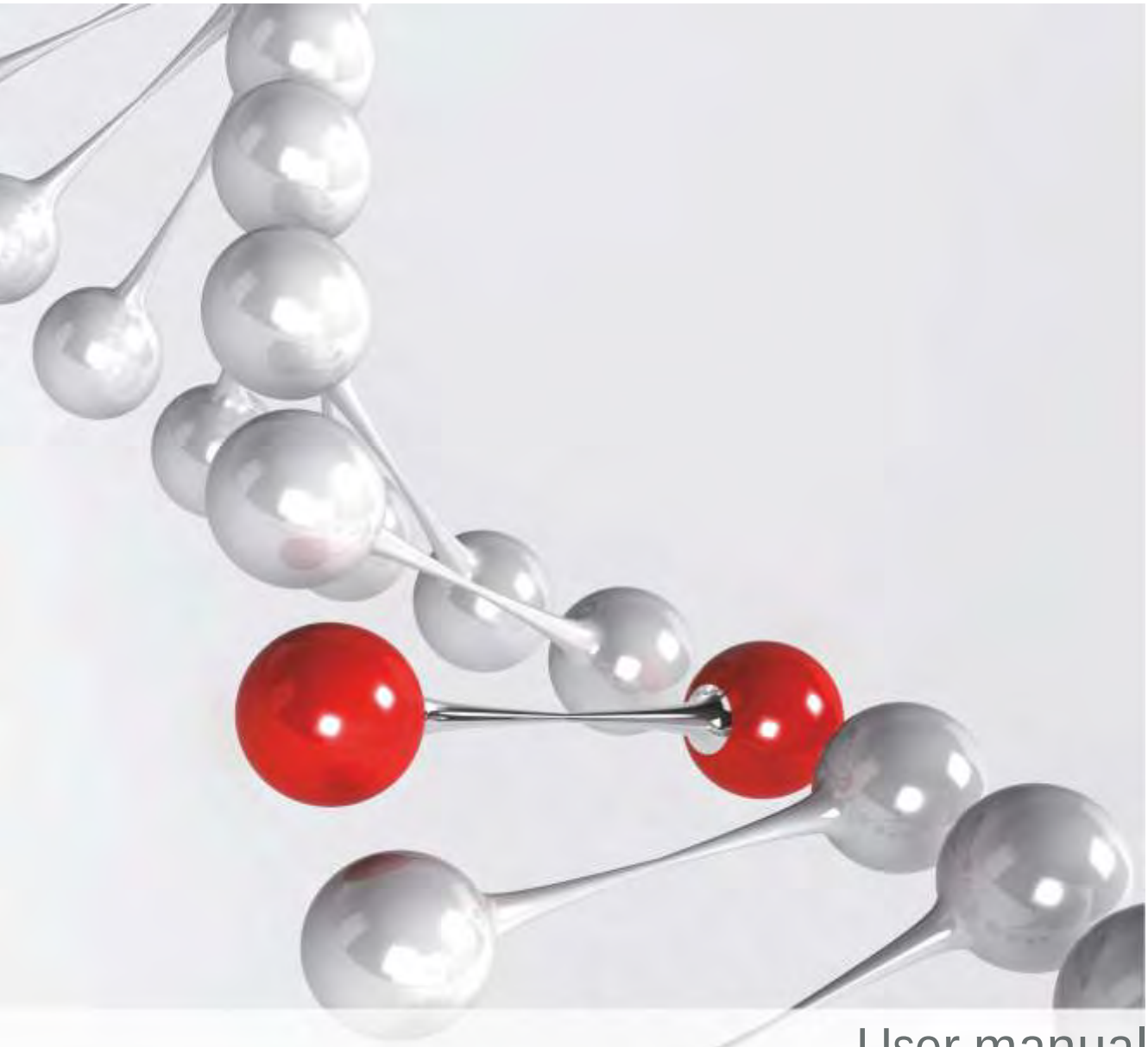


User manual
Invisorb® DNA Swab HTS 96 Kit / STARlet

for use on the STARlet platform from Hamilton®

For walk-away automated DNA isolation and purification from 200 µl of stabilized swab samples and 50 µl of stabilized saliva samples

REF 7135330400

STRATEC Molecular GmbH, D-13125 Berlin

Instruction for Invisorb® DNA Swab HTS 96 Kit/ STARlet

The **Invisorb® DNA Swab 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 stabilized Swab samples (200 µl stabilized sample) and stabilized saliva samples (50 µl stabilized sample).

The interplay of the nucleic acid extraction and purification chemistry provided by the

Invisorb® DNA Swab HTS 96 Kit/ STARlet was intensely tested and validated.

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.

For research use only!

Table of content

Kit contents of Invisorb® DNA Swab HTS 96 Kit/ STARlet

2 ml Sarstedt Screw Tubes are necessary for preparation!

	4 x 96 extractions	24 x 96 extractions
Catalog No.	7135330300	7135330400
Lysis Buffer HLT	2 x 80 ml	3 x 250 ml
Proteinase S	4 x 2 ml	24 x 2 ml
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 200 ml)	empty bottle (final volume 1000 ml)
Wash Buffer HLT	270 ml (final volume 450 ml)	4 x 360 ml (final volume 4 x 600 ml)
Wash Buffer II	150 ml (final volume 500 ml)	5 x 300 ml (final volume 5 x 1000 ml)
Elution Buffer M	500 ml	1000 ml
2.0 ml Collection Plate	4	6 x 4
Elution Plate L	4	24
DNA Binding Plate D	4	6 x 4
Sealing Foils	8	48
Initial steps	Fill 200 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 180 ml 99.7% Isopropanol to the bottle **Wash Buffer HLT.** Add 350 ml of 96-100% Ethanol to the bottle **Wash Buffer II.** Mix thoroughly and always keep the bottles firmly closed! Vortex Proteinase S before transferring the needed amount into the STARlet.	Fill 1000 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 240 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! Vortex Proteinase S before transferring the needed amount into the STARlet.

Content of SalivaGene Collection Sets

SalivaGene Collection Sets	50 pieces	
SalivaGene Buccal Swab	1035230200	1035230200 (8x50 pieces)
SalivaGene Collection Module II	1035212200	1035212300 (125 pieces)
SalivaGene Collector	1035211200	
SalivaGene Swab Comfort	1035231200	1035231300 (300 pieces)

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® DNA Swab HTS 96 Kit/ STARlet,** 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).

Wash Buffers charged with ethanol or isopropanol should be appropriately sealed and stored at room temperature.

Quality Control and product warranty

STRATEC Molecular guarantees the correct function of the **Invisorb® DNA Swab 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® DNA Swab 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.

In case of questions or problems regarding any aspects of **Invisorb® DNA Swab 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)30-9489-2903/ 2907 or contact your local distributor.

Intended use

The **Invisorb® DNA Swab HTS 96 Kit/ STARlet** has been designed for fully automated extraction and purification of genomic DNA and 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 plasticware besides components obtainable by Hamilton Inc.(as filter-tips and reagent trays)) necessary for preparation of total NA are provided by the **Invisorb® DNA Swab HTS 96 Kit/ STARlet** .

The nucleic acid isolation protocol is suitable for routinely walk-away automated preparation of DNA from 200 µl of stabilized swab sample material. Targets are nucleic acids from genomic and bacterial DNA. For efficient extraction, an appropriate sample storage is essential (see “Sampling and storage of the starting material”, page 13).

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, if desired), allowing safe handling of potentially infectious samples.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA 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, implied or expressed.

The user is responsible to validate the performance of the STRATEC Molecular Product for any particular use. STRATEC Molecular does not provide 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 not suitable 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® DNA Swab 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 accordingly to local safety regulations.

Subsequently European Community risk and safety phrases for the components of the **Invisorb® DNA Swab HTS 96 Kit/ STARlet,** to which they apply, are listed.

Lysis Buffer HLT

Warning

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

Proteinase S-Tube (Proteinase S)

Danger

H317-H318-P280-P305+P351+P338

H302: Harmful if swallowed.
H315: Causes skin irritation.
H317: May cause an allergic skin reaction.
H318: Causes serious eye damage.
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 Invisorb® DNA Swab HTS 96 Kit/ STARlet

The **Invisorb® DNA Swab HTS 96 Kit/ STARlet** is the ideal tool for an efficient and fully automated DNA extraction and purification from stabilized Swab samples with the STARlet, a Hamilton® robotic platform. The **Invisorb® DNA Swab HTS 96 Kit/ STARlet** provide several key features: high recovery rates for low nucleic acid amounts, high reproducibility, complete automation without the need of additional manual preparations, direct processing of primary tubes and an optimal utilization of 96 well plate capacities. 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**
Stabilized Swabs (200 µl) Stabilized Saliva (50 µl) Rinsed Swabs (200 µl)	depending on sample (storage and source) ***Note:*** Quantitative (RT)-PCR is recommended for determination of the DNA yield	about 100 min for 96 samples

The Starlet instrument uses an 8 channel pipettor to transfer samples and buffers and a vacuum station to separate the DNA during the various extraction phases: lysis-binding-washing-elution.

After a sample specific lysis, using **Lysis Buffer HLT** and **Proteinase S,** binding conditions are adjusted upon addition of **Binding Solution.** The genomic/ bacterial DNA binds to DNA Binding Plate D 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® DNA Swab HTS 96 Kit/ STARlet.**

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 such as 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.

PSP®-Treatment with SalivaGene Collector

Contents:
SalivaGene funnel, lid, tube, each tube contains approx. 150 mg of SalivaGene reagent.

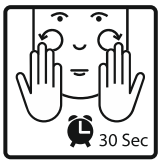
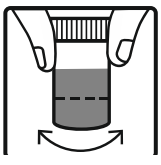
Limitations of the procedure:
Do not eat, drink, smoke or chew gum at least 30 min prior to saliva collection. Collecting 2 ml of saliva may require several minutes. Putting some grains of sugar on the tongue helps to produce saliva and does not interfere with the stabilization.

1. 	Remove seal from tube completely, and discard the seal or unscrew lid from tube and put it aside for further use.
2. 	Insert funnel into tube tightly.
3. 	Rub cheeks against teeth intensely for 30 sec.
4. 	Collect saliva to indicated fill level, avoid making and measuring air bubbles.
5. 	Remove and discard the funnel. Press lid firmly on the tube until it clicks or screw lid tightly onto the tube again.
6. 	Shake tube for 15 sec to dissolve white reagent.
7. 	Store the tube upright for 2-20 min with occasional shaking until SalivaGene reagent is dissolved. Some cloudy material may occur during this process. This does not interfere with stabilization.
8. 	For barcode sample tracking stick the small barcode tape vertically onto the tube

PSP®-Treatment with SalivaGene Collection Module II

Contents:
Collection tube, Stabilizer tube. The stabilizer tube contains approx. 2 ml of liquid Saliva DNA Stabilizer reagent.

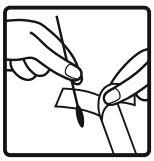
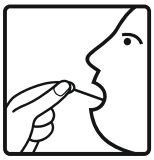
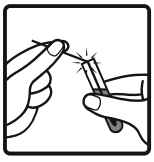
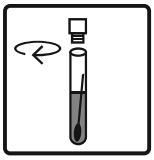
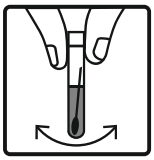
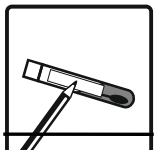
Limitations of the procedure:
Do not eat, drink, smoke or chew gum at least 30 min prior to saliva collection. Collecting 2 ml of saliva may require several minutes. Putting some grains of sugar on the tongue helps to produce saliva and does not interfere with the stabilization.

1. 	Unscrew lid from Collection tube and put it aside for further use.
2. 	Rub cheeks against teeth intensely for 30 sec.
3. 	Collect saliva to indicated fill level, avoid making and measuring air bubbles.
4. 	Unscrew and discard lid from Stabilizer tube. Pour Saliva DNA Stabilizer reagent into Collection tube.
5. 	Screw lid tightly onto the Collection tube again.
6. 	Shake tube for 15 sec to mix saliva and Saliva DNA Stabilizer reagent.

PSP®-Treatment of SalivaGene Buccal Swab

Contents:
Swab, Stabilizer tube, label for donor description. The Stabilizer tube contains approx. 1 ml of liquid DNA Stabilizer reagent.

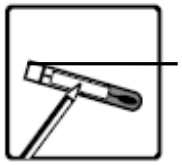
Limitations of the procedure:
Do not eat, drink, smoke or chew gum at least 30 min prior to swab collection. Ensure the swab tip does not come into contact with any surface prior to collection. Put the Stabilizer tube upright to prevent the liquid inside the tube from spilling. Be sure to move the swab over the entire cheek and to moisten it with saliva.

1. 	Open swab package and remove swab without touching the tip.
2. 	Insert swab into the mouth and rub swab tip firmly against the inner cheeks for about 30 seconds on each side.
3. 	Unscrew lid from Stabilizer tube and put it aside for further use. Insert swab into the Stabilizer tube.
4. 	Break swab tip at breaking point. Discard the broken handle part.
5. 	Screw lid tightly onto the Stabilizer tube again.
6. 	Shake tube for 15 sec to mix buccal cells and DNA Stabilizer reagent.
7. 	Label Stabilizer tube with donor name and collection date using provided label.

PSP®-Treatment of SalivaGene Swab Comfort

Contents:
Swab, Stabilizer tube, label for donor description. The Stabilizer tube contains approx.650 µl of liquid DNA Stabilizer reagent.

Limitations of the procedure:
Do not eat, drink, smoke or chew gum at least 30 min prior to swab collection. Ensure the swab tip does not come into contact with any surface prior to collection. Put the Stabilizer tube upright to prevent the liquid inside the tube from spilling. Be sure to move the swab over the entire cheek and to moisten it with saliva.

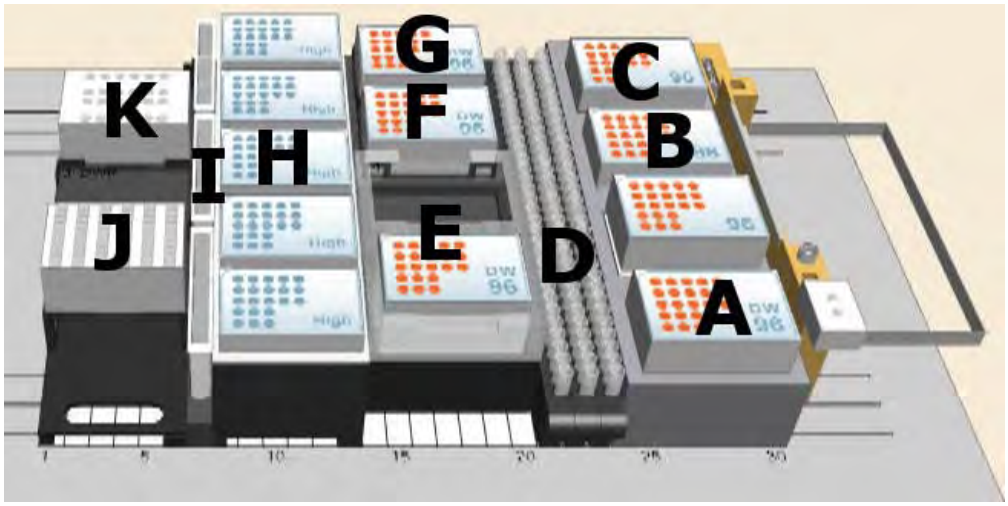
1. 	Open swab package and remove swab without touching the tip.
2. 	Insert swab into the mouth and rub swab tip firmly against the inner cheeks for about 30 seconds on each side.
3. 	Unscrew lid from Stabilizer tube and put it aside for further use. Insert swab into the Stabilizer tube.
4. 	Break swab at breaking point. Discard the broken handle part.
5. 	Screw lid tightly onto the Stabilizer tube again.
6. 	Shake tube for 15 sec to mix buccal cells and DNA Stabilizer reagent.
7. 	Label Stabilizer tube with donor name and collection date using provided label.

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 we can assure that the 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) and F (Elution Plate) comprise the three 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 L on position E

Principle and procedure

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

- sample preparation if required
- lysis and protein digestion
- binding of the DNA to the membrane of the filter plate
- washing the membrane bound DNA
- evaporation of alcohol
- elution of DNA

After lysis the DNA binds to the filter plate whereas contaminations and enzyme inhibitors are efficiently removed during the following wash steps while purified DNA is eluted in
Elution Buffer M.

Pretreatment of the Sample Tubes: It leads to better and equal results if the sample tubes with the swabs in it were placed on a shaker before preparing them.

Procedure

Lysis
Samples are lysed in the 2.0 ml Collection Plate at elevated temperatures in the presence of **Lysis Buffer HLT** and **Proteinase S**

Binding of the genomic DNA
After addition of **Binding Solution** to the lysate, the DNA is bound to the filter plate.

Removing residual contaminants
Contaminants are efficiently removed while the DNA remains bound to the filter plate.

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

Yield and quality of pathogen DNA

The amount of purified pathogen DNA in the **Invisorb® DNA Swab HTS 96 Kit/ STARlet** procedure depends on the sample type, the bacterial titer, sample source, transport, storage, and age of the sample.

Yield and quality of isolated pathogen DNA are suitable for any molecular-diagnostic detection system.

Different amplification systems vary in efficiency depending on the total amount of nucleic acids present in the reaction. Eluates from this kit contains genomic and/or pathogen DNA.

Quantitative RT-PCR is recommended for determination of DNA yield.

The kit is suitable for downstream analysis with NAT techniques, such as PCR**, qPCR, RT-qPCR, LAMP, LCR. Diagnostic assays should be performed accordingly to the manufacturer's instructions.

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, and correct quantities. If there are any unconformities, notify STRATEC Molecular immediately in writing with the immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information’s” (see page 7). Do not use damaged kit components because 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:
Fill 200 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 180 ml 99.7% Isopropanol to the bottle **Wash Buffer HLT.** Add 350 ml of 96-100% ethanol to the bottle **Wash Buffer II.** Mix thoroughly and always keep the bottles firmly closed!
24 x 96 extractions:
Fill 1000 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 240 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 for the kit

- Measuring cylinder (250 ml, 1000ml)
- Conductive Tips with Filter, 1ml
- Disposable gloves
- ddH_2O
- Vortex
- 96-100% ethanol
- Isopropanol * (molecular biological grade)
- 2 ml Sarstedt Screw Tubes are necessary for preparation!

*The **Invisorb® DNA Swab 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

Hamilton equipment to be supplied by the customer

The **Invisorb® DNA Swab 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
- CO-RE gripper with attachment for waste block; 188066APE
- Conductive Filter Tips

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

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

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

Sampling and storage

For reproducible and high yields the 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.

Saliva:

The protocols work with aliquots of transport media for collection of fresh saliva or swab samples. The DNA of fresh saliva (collected using the **SalivaGene Collector** or **SalivaGene Collection Module II**) is stable for at least one year at room temperature in the stabilization buffer.

Swab:

The DNA of swab samples (collected using the **SalivaGene Buccal Swab** or **SalivaGene Swab Comfort**) is stable for at least 6 months at room temperature in the stabilization buffer. Long-term storage (≥ 6 month) of the sample should be done at - 20°C after receiving the collection device. Please, before freezing the sample squeeze and remove the swab.

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

Preparation of starting materials

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. 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 and incubated at RT or 37°C for 15mins.*

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 it 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 the 2.0 ml Collection Plate and incubated at RT or 37°C.*

Preparing and loading of the Hamilton® system

Preparing the reagents

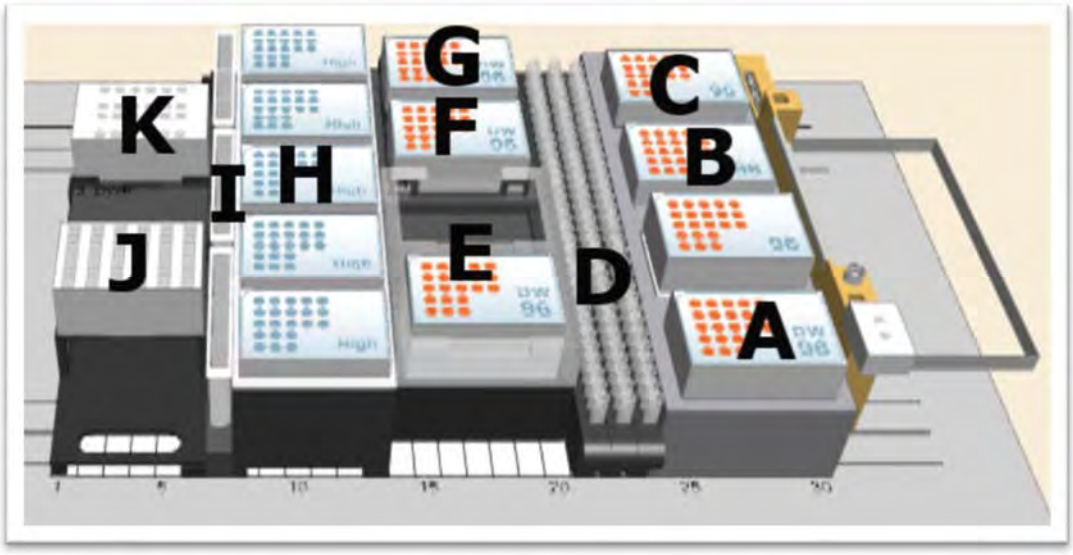
Before starting a new kit, add either ethanol or isopropanol to the corresponding Wash Buffers (Check tags on the bottles).

Before starting a new run

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 have a different deck layout and needs different positioning.



The 96-well Filter Plate can be used sequential (max 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 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 Deep Well Plate on the **heater-shaker (A)** and one 96 Elution Plate L on the **position F**. After running the assay the eluates are located in the Elution Plate in the **Vacuum Station on position E**.

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

Place a small piece of paper-tissue on **position C** for easier cleanup 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 for a proper sample transfer.

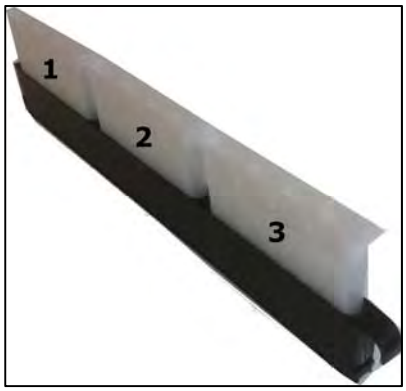
very important: ***Please avoid foam formation when handling with buffers. If foam is present in a container, the machine may not detect the buffer level correctly.***

PLEASE CHECK THE SUGGESTED AND MAXIMUM FILL VOLUMES CALCULATED BY THE ASSAY

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

Place the Tray Holder on **position J**

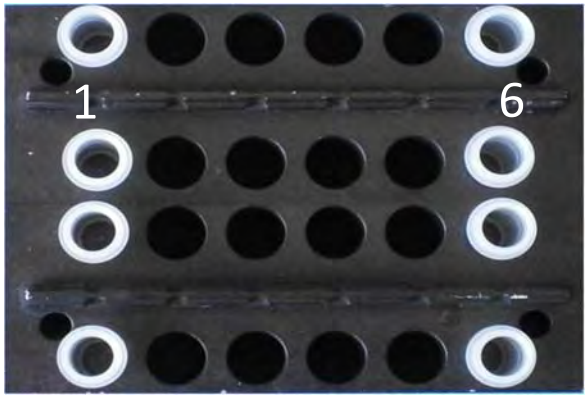
Load Wash Buffer HLT and Wash Buffer into the larger reagent trays referring to the sequences: Wash Buffer HLT in tray no.: 1; Wash Buffer in tray no.: 2 and 3.



Place the Tray Holder on **position I**

Take empty tubes and fill them with the calculated Proteinase S volume in position **1** (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 that it is clean and dry before doing a new run.

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 that the sealing gasket of the Vacuum station can be seen by pressing firmly on the Filter-Plate to ensure correct Vacuum during the filtration process.

Place the filter-tips on the corresponding tip-sequences in **position H**. The **Invisorb® DNA Swab HTS 96 Kit/ STARlet** 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® DNA Swab HTS 96 Kit/ STARlet**

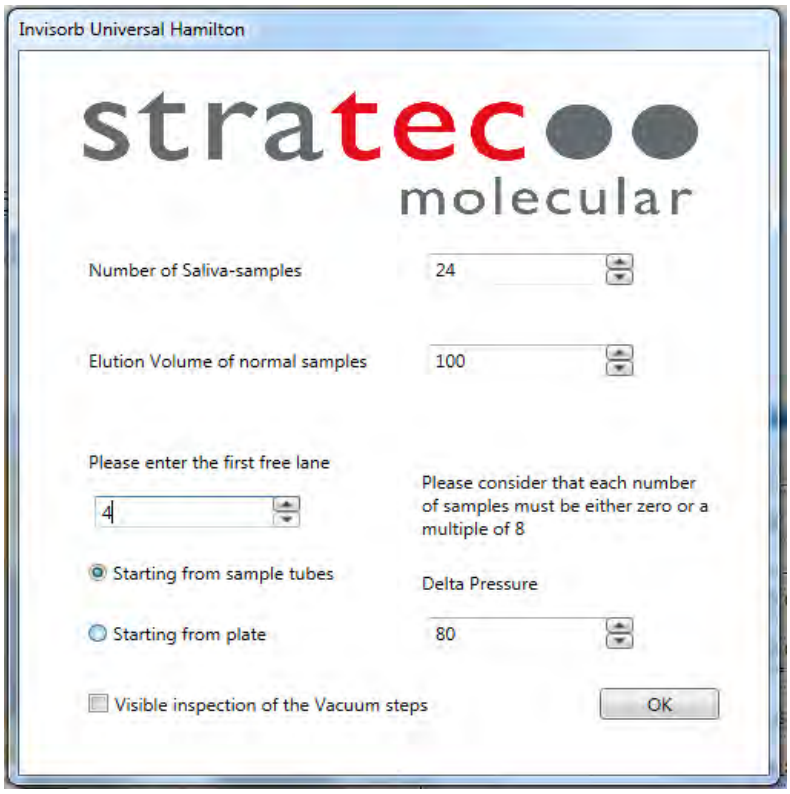
Press on Run Control to start the machine.

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.

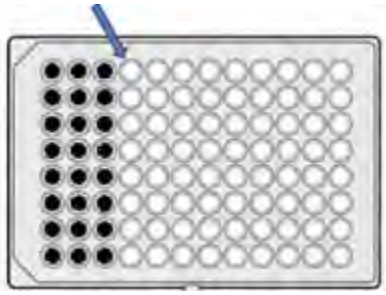
Additionally, the instrument will ask for:

- number of samples
- Elution volume
- for the first free lane on the Collection Plate,
- if the run should start from sample tubes or directly from plate,
- if the Filter Plate should be inspected after the vacuum steps and
- the delta pressure (default 80 mbar) is ok.



Example: Here, 24 samples are shown. The samples will be eluted in 100 µl elution volume. The run will start from the 4th lane of the plate, starting from sample tubes and it is not intended to inspect the Filter Plate after the first two vacuum steps.

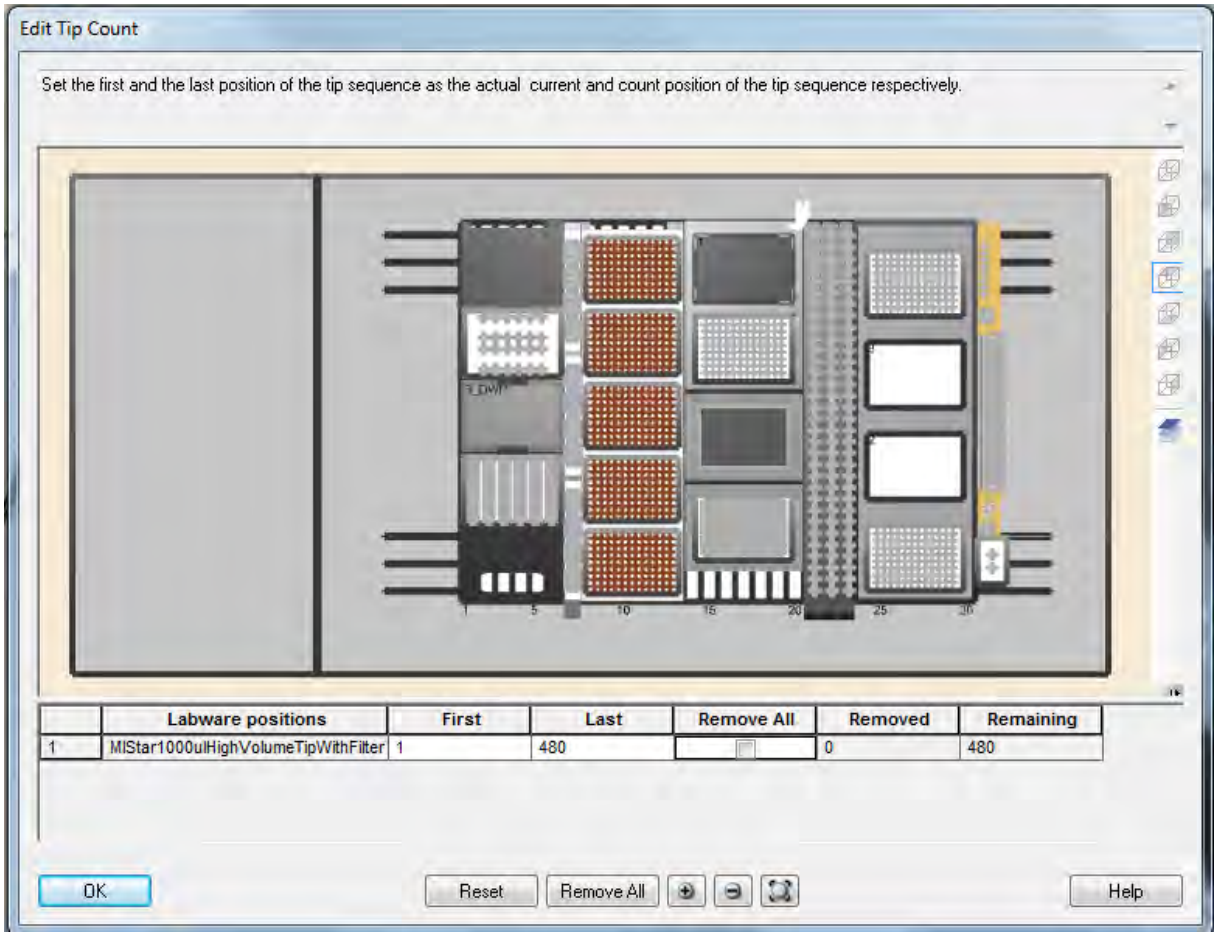
3 used lanes - lane 4 is the first free lane



If the button to inspect the Filter Plate vacuum steps is pressed, ensure to watch the Hamilton Starlet or at least be present in the room to inspect the plate when the acoustic

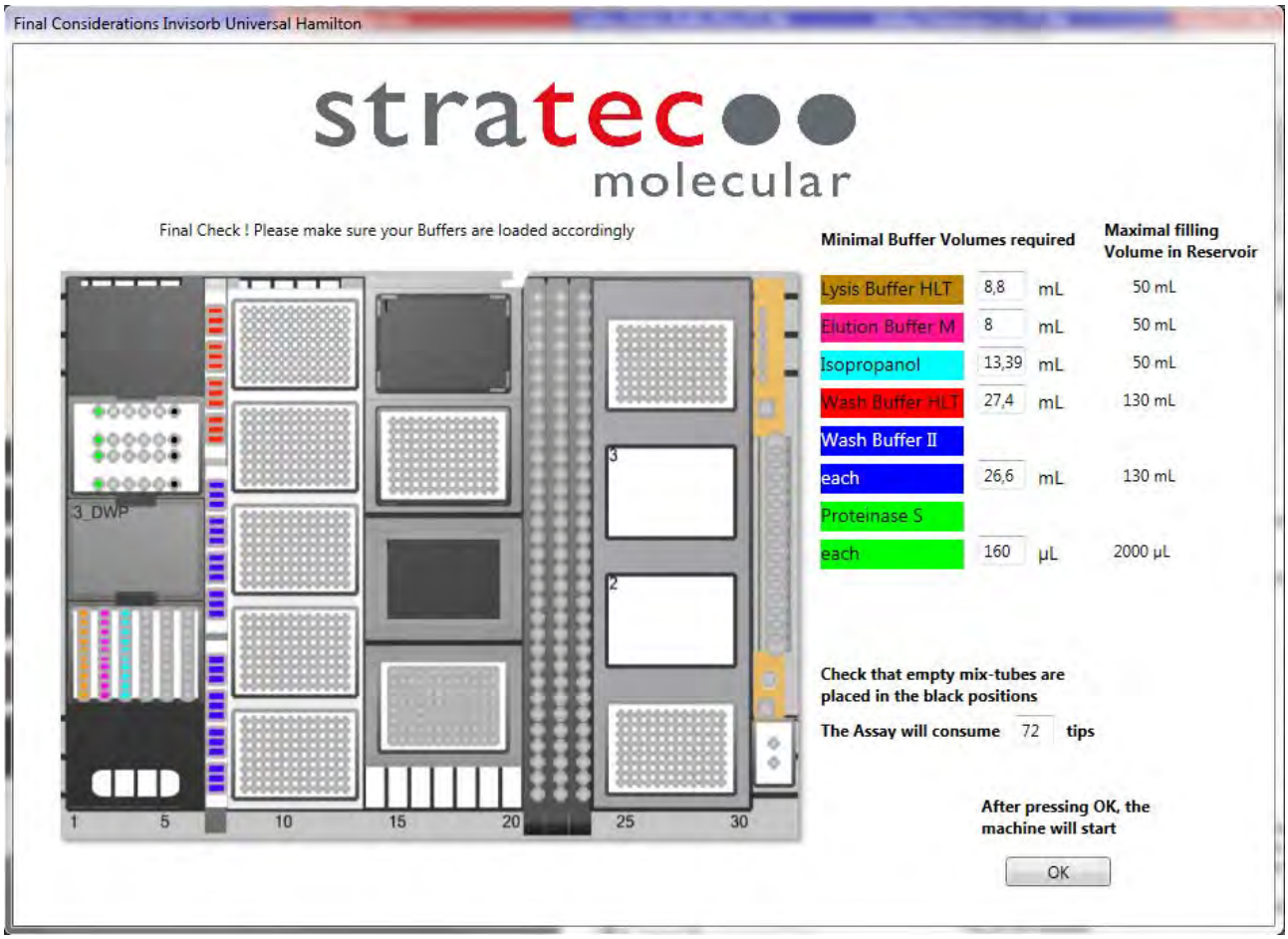
signal for inspection sounds and continue the assay if every well is empty. <u>Please be quick.</u> In case of too long waiting time, the assay performance will be decreased.

Next, the appropriate 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.

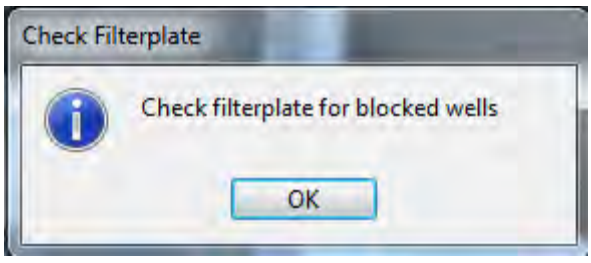
Pressing the button “OK” will switch to the next step.



In this picture all buffers and amounts are displayed that are required for the number of selected samples (in this case 24 samples). Fill the containers with minimal the required buffer volumes (do not exceed maximal filling volume) and check that the buffers are located at the exact corresponding position.

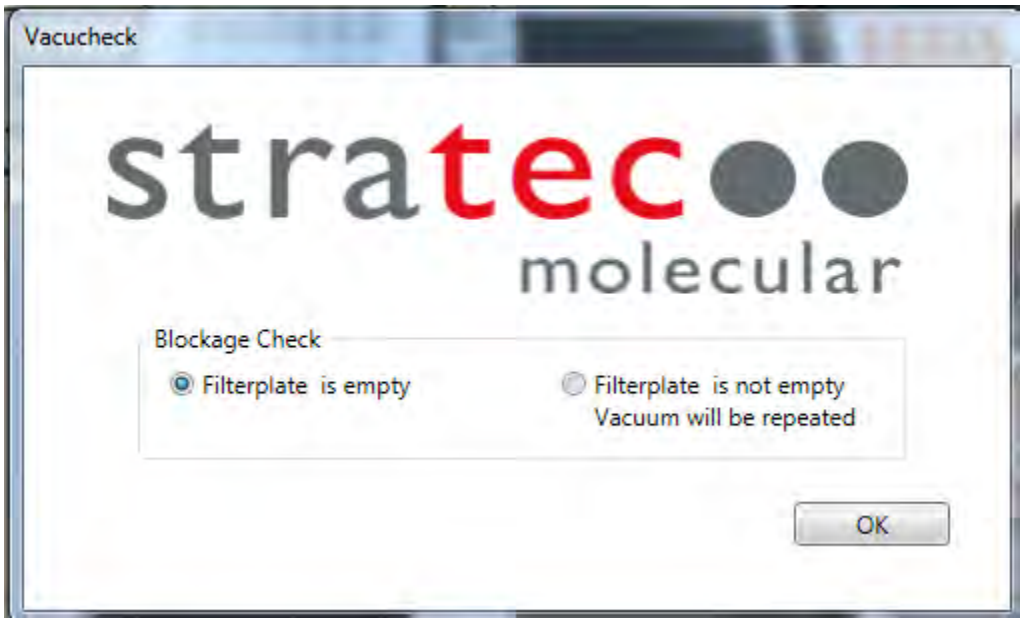
After pressing the button “OK”, the assay will be performed without any user-interference if no visible inspection after the vacuum steps was selected.

If a visible inspection should be performed, an acoustic signal 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.

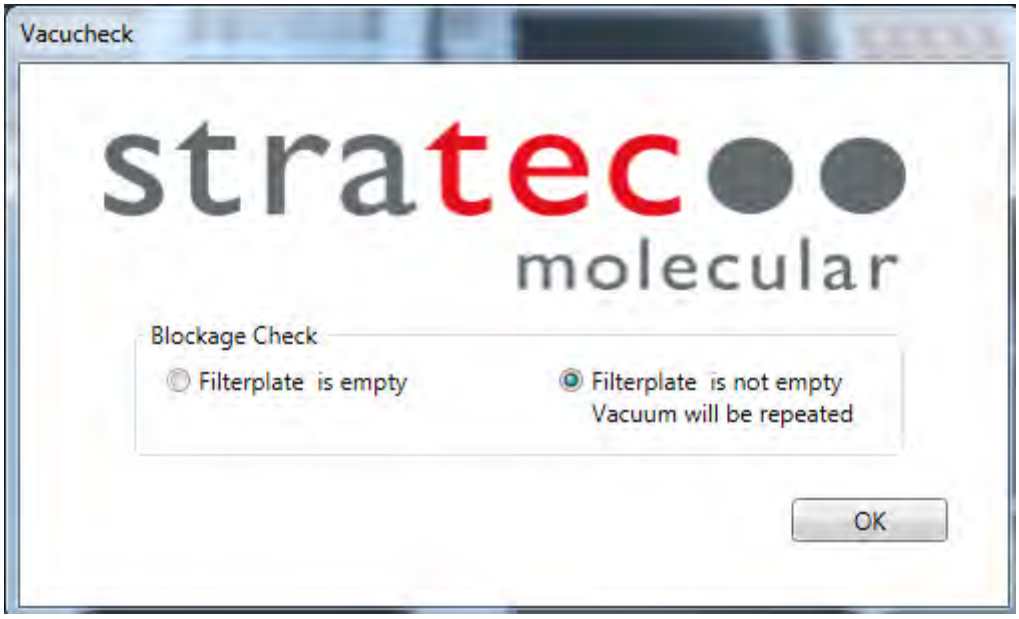


Pressing the button “OK” will end the acoustic signal.

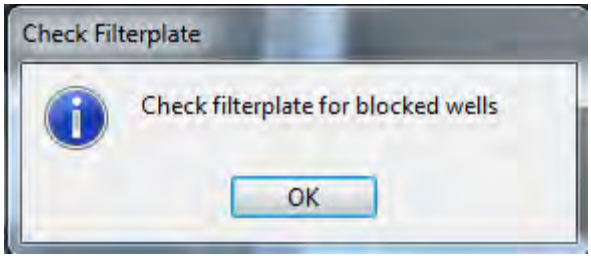
The machine is stopped. Inspecting the filter-plate for blocked wells is possible at this point.



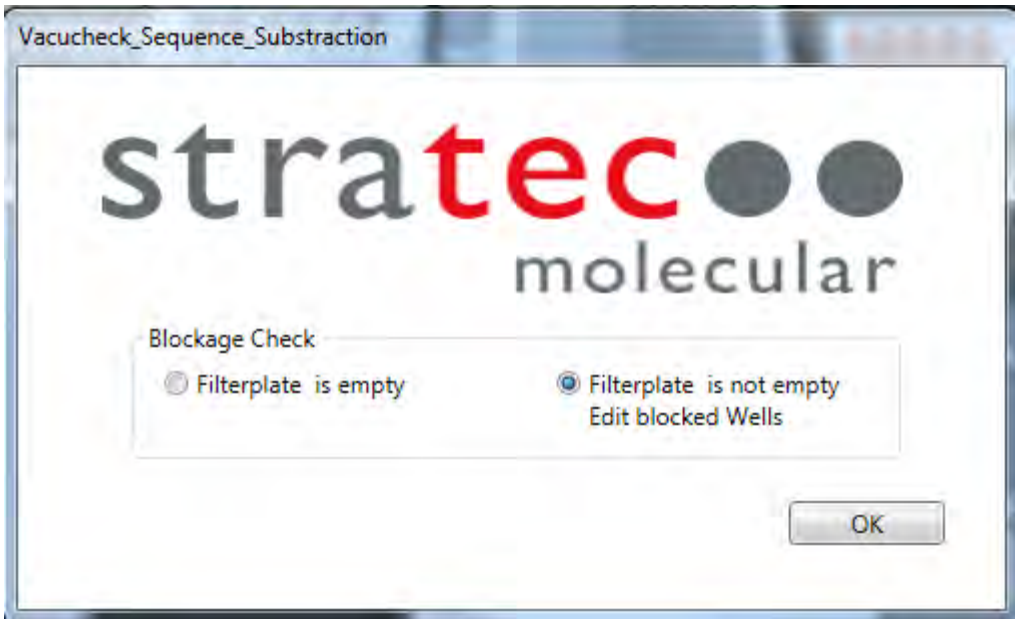
If the field “filter-plate is empty” is selected and the button “OK” is pressed, the assay will continue and asks again after the second vacuum step. If everything looks good, click the button “OK” again and the assay will continue until the end without any additional user interaction.



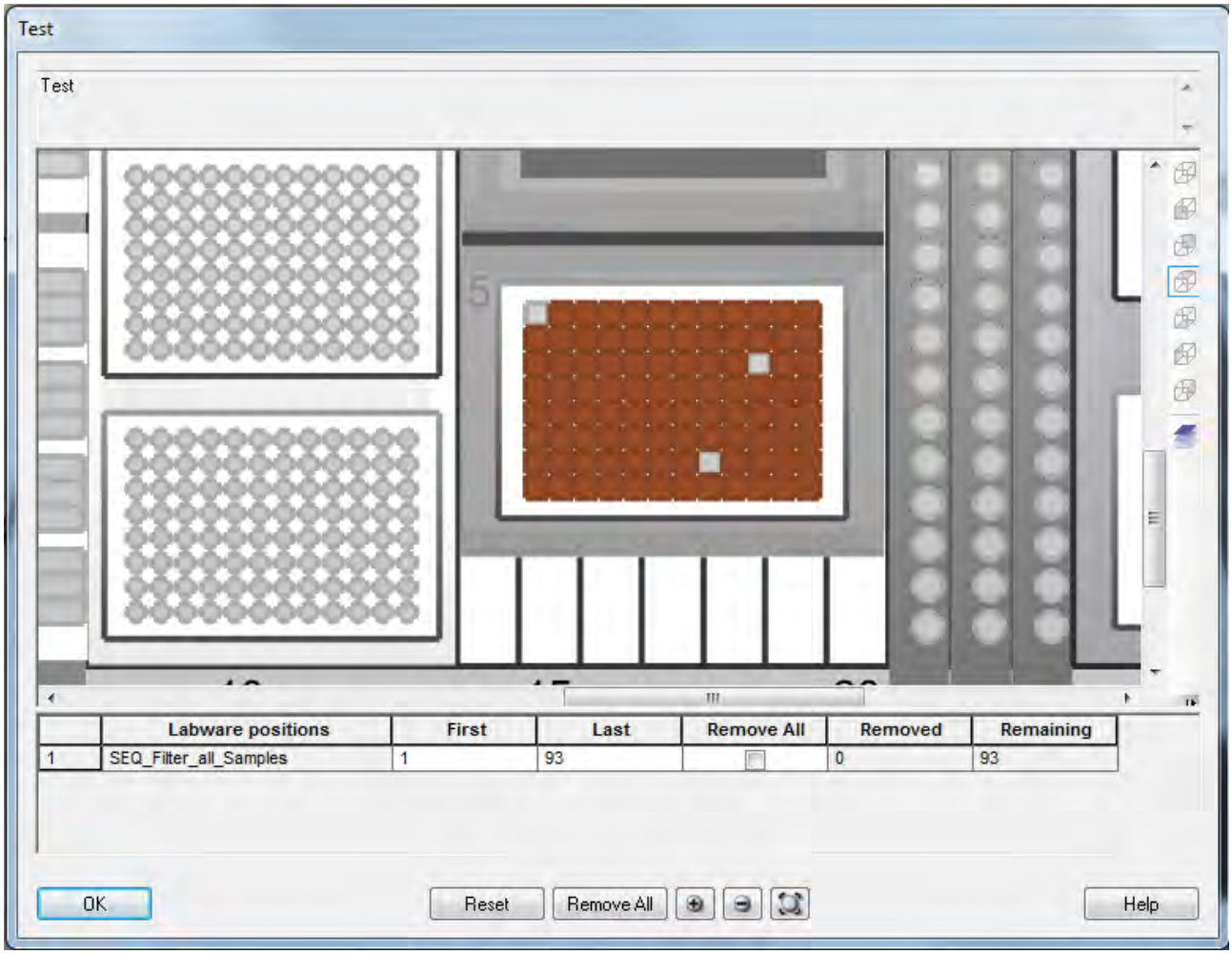
If the field “filter-plate is not empty” is selected, another vacuum step will be performed.



Stop the acoustic signal by pressing the button “OK”.



If the field “filter-plate is not empty” and “OK” is selected, the following picture will show up:



Mark the blocked wells on the computer like shown above and press the “OK” button. (In this case Well A1, G8 and C10 are blocked) The assay will continue and ask again after the second vacuum step. If everything looks good (except maybe previously blocked wells), press the button “OK”. The assay will run until the end without any further user interaction.

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

After a run

The Elution Plate with the eluates will be located at the vacuum-station position.

Transfer the eluates from the Elution Plate into a suitable storage solution or seal the Elution Plate.

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.

TAKE OUT THE RUBBER SEALS BEFORE SWITCHING ON THE UV-LIGHT AS IT WILL AGE AND LOOSE ITS FUNCTION UNDER UV-LIGHT.

The rubber seal of the Plate Holder for the Vacuum Station needs to be groomed with oil or glycerol from time to time.

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. 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) 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	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 Ensure that the **Wash Buffers** are equilibrated at room temperature

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.

Ordering information

Product	Package Size	Order No.
Invisorb® DNA Swab HTS 96 Kit/ STARlet	24 x 96 preparations	7135330400

Related Products	Package Size	Order No.
InviMag® SalivaGene DNA Kit/ IG	8 x 12 preps	2435260100
InviMag® SalivaGene DNA Kit/ KF96	1 x 96 preparations	7435060100
InviMag® SalivaGene DNA Kit/ KF96	5 x 96 preparations	7435060200
PSP® SalivaGene DNA HTS 96 Kit/ C	2 x 96 preparations	7035360200
PSP® SalivaGene DNA HTS 96 Kit/ C	4 x 96 preparations	7035360300
PSP® SalivaGene DNA HTS 96 Kit/ C	24 x 96 preparations	7035360400
PSP® SalivaGene DNA Kit	50 preparations	1035200200
PSP® SalivaGene DNA Kit	250 preparations	1035200300
SalivaGene Swab Comfort	50 pieces	1035231200
SalivaGene Swab Comfort	300 pieces	1035231300
SalivaGene Collector	50 pieces	1035211200
SalivaGene Collection Module II	50 container	1035212200
SalivaGene Collection Module II	125 container	1035212300
SalivaGene Buccal Swab	50 pieces	1035230200
SalivaGene Buccal Swab	8x 50 pieces	1035230200

Possible suppliers for Isopropanol:

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order Nr. 6752

Applichem
2-Propanol
Order Nr. A3928

Sigma
2-Propanol
Order Nr. 59304-1L-F

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