

## User manual

# InviMag<sup>®</sup> Universal Kit/ IG

for use on the InviGenius<sup>®</sup> and InviGenius<sup>®</sup> PLUS, STRATEC Molecular GmbH

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



IVD

REF

2450120100



STRATEC Molecular GmbH, D-13125 Berlin

## Instruction for InviMag<sup>®</sup> Universal Kit/ IG

The **InviMag<sup>®</sup> Universal Kit/ IG** combines the advantages of the innovative InviMag<sup>®</sup> technology with easy handling of magnetic particles of high purity in combination with the InviGenius<sup>®</sup> robotic platform.

The **InviMag<sup>®</sup> Universal Kit/ IG** in combination with the InviGenius<sup>®</sup> is the ideal tool for a walk-away automated isolation of highly pure total (genomic, bacterial, and viral) DNA and of viral RNA from 200 µl blood, serum, plasma, cerebrospinal fluid, cell culture supernatants and other cell free body fluids, like urine as well as from swabs (rinsed liquid), sputum, BAL or supernatant from stool suspension for *in-vitro* diagnostic purposes.

The nucleic-acid-binding magnetic particles are characterized by a specific surface, a uniform size distribution and good suspension stability.

The **InviGenius<sup>®</sup>** is a compact walk-away DNA/RNA extraction platform with full in-process control, including the following modules e.g. like a pipettor, heat incubator, barcode reader, magnet tool, PC and touch screen, barcode labelled sample racks for primary tubes and barcode labelled reagent racks, which helps to deliver premium quality nucleic acid for routine laboratories by eliminating human errors, standardizing the extraction process, and offering an integrated solution for data storage, backup and archiving using unique bar codes for samples and reagents to avoid unwanted transpositions.

Due to the high purity, the isolated DNA / RNA is ready to use for a broad panel downstream applications or can be stored at –20°C for subsequent use.



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

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

Trademarks: InviMag<sup>®</sup>, Invisorb<sup>®</sup>, InviGenius<sup>®</sup> Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

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

InviMag<sup>®</sup>, Invisorb<sup>®</sup> and InviGenius<sup>®</sup> are registered trademarks of STRATEC Biomedical AG.

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

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## Kit contents of InviMag® Universal Kit/ IG

Component	4 x 12 reactions	8 x 12 reactions	Reagent sufficient for
<b>Catalogue No.</b>	2450120000	2450120100	
<b>Lysis Buffer HLT</b>	50 ml	50 ml	96 samples (in max. 16 runs)
<b>PKC tube</b> (Proteinase K / Carrier-RNA)	for 4 x 800 µl working solution	for 8 x 800 µl working solution	12 samples (in max. 2 runs)
<b>MAP Solution B/ IG</b>	2.6 ml	2 x 2.6 ml	48 samples (in max. 8 runs)
<b>Binding Solution</b> (fill with 99.7% Isopropanol)	empty bottle (final volume 80 ml)	empty bottle (final volume 80 ml)	96 samples (in max 16 runs)
<b>Wash Buffer II</b>	36 ml (final volume 120 ml)	2 x 36 ml (final volume 2 x 120 ml)	48 samples (in max. 8 runs)
<b>Elution Buffer M</b>	80 ml	80 ml	96 samples
<b>RNase-free Water</b>	15 ml	15 ml	
<b>Incubation Plate A</b>	1	1	8 runs per plate
<b>Working Plate A</b>	2	4	2 runs per plate
<b>Elution Plate E</b>	1	1	8 runs per plate
<b>Microtube Caps</b>	8	8	
<b>Sheath Box</b>	1 (2 racks á 48 sheaths)	1 (2 racks á 48 sheaths)	4 runs per plate
<b>Sealing Foils</b>	4	4	
<b>Incubator Stripe Foils</b>	2	2	
<b>Initial steps</b>	<p>Resuspend lyophilized material in <b>PKC Tube</b> by addition of 800 µl <b>RNase-free Water</b> (included) and mix thoroughly!</p> <p>Add 80 ml of <b>99.7% Isopropanol</b> (molecular biologic grade) into the empty bottle labelled <b>Binding Solution</b></p> <p>Add 84 ml of 96-100% ethanol to the bottle <b>Wash Buffer II</b>. Mix thoroughly and always keep the bottle firmly closed!</p>	<p>Resuspend lyophilized material in <b>PKC Tube</b> by addition of 800 µl <b>RNase-free Water</b> (included) and mix thoroughly!</p> <p>Add 80 ml of <b>99.7% Isopropanol</b> (molecular biologic grade) into the empty bottle labelled <b>Binding Solution</b></p> <p>Add 84 ml of 96-100% ethanol to each bottle <b>Wash Buffer II</b>. Mix thoroughly and always keep the bottle firmly closed!</p>	

## Symbols



Manufacturer

LOT

Lot number

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

REF

Catalogue number



Expiry date



Consult operating instructions



Temperature limitation



Do not reuse



Humidity limitation

## Storage

All buffers and kit contents of the **InviMag® Universal Kit/ IG**, except the **dissolved PKC-Tube** 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 Buffer** charged with ethanol should be appropriately sealed and stored at room temperature.

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

**PKC Tube:** PKC Tubes with dissolved enzyme mixture must be stored at -20°C. So the dissolved enzyme mix is stable as indicated on the kit package.

## Quality Control and product warranty

STRATEC Molecular warrants the correct function of the **InviMag® Universal Kit/ IG** for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a lot connected problem, the product will be replaced free of charge.

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

In accordance with STRATEC Molecular's EN ISO 9001 and EN ISO 13485 certified Quality Management System the performance of all components of the **InviMag® Universal Kit/ IG** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

In case of any questions or problems regarding any aspects of **InviMag® Universal Kit/ IG** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

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

from Germany: +49-(0)30-9489-2901/ 2910

from abroad: +49-(0)30-9489-2907

or contact your local distributor.

## Intended use

The **InviMag® Universal Kit/ IG** is designed for fully automated extraction and purification of DNA and/or RNA from 12 samples per run by using magnetic beads in combination with the InviGenius® system. Common blood collection tubes can be used to assemble a set of samples (diameter 12-17 mm). All utilities (reagents and plastics except filter tips) necessary for preparation of nucleic acids are provided by the **InviMag® Universal Kit/ IG**.

The nucleic acid isolation protocol is suitable for routinely walk-away automated simultaneous preparation of high quality genomic, bacterial and viral DNA as well as viral RNA from fresh or frozen samples like human blood\*, serum\*, plasma\*, cerebrospinal fluid, cell culture supernatants and other cell free body fluids, rinsed liquid from swabs, urine, supernatant from stool suspension, BAL, sputum etc.. For an efficient extraction the appropriate sample storage is essential (see "Sampling and storage of the starting material", page 8).

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

The procedure of the **InviMag® Universal Kit/ IG** is optimized for the isolation of nucleic acids from up to 200 µl starting material. However, we advise to provide at least 550 µl sample per primary tube to prevent pipetting distribution errors (more if the tube diameter is bigger than 12 mm). The final processed sample volume is 200 µl.

THE PRODUCT IS INTENDED FOR USE BY PROFESSIONALS SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA/ RNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings. To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

*The kit is in compliance with EU Directive 98/79/EC on in vitro medical devices. But it is not applicable for in-vitro diagnostic analysis in countries where the EU Directive 98/79/EC on in-vitro medical devices is not recognized.*

## Product use limitation

The kit is validated for e.g. viral DNA/RNA extraction from cell-free body fluids and rinsed liquids, specifically for human serum and plasma. Related applications will need a separate validation. Extraction of other than human DNA from blood or of total RNA has not been evaluated with this kit.

Differing of starting material may lead to inoperability. Therefore, neither a warranty nor guarantee in this case will be given, implied or expressed. The included chemicals are only useable once.

The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide validations 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 plastics are for laboratory use only. They must be stored in the laboratory and must not be used for other purposes 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.stratec.com](http://www.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 **InviMag® Universal Kit/ IG** 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 **InviMag® Universal Kit/ IG** to which they apply, are listed.

### Lysis Buffer HLT



Warning

H302-H315-H319, P280-P05+P351+P338

### Proteinase K



Danger

H315-H319-H334-H335-P280-305-351-338

H302: Harmful if swallowed.

H315: Causes skin irritation.

H319: Causes serious eye irritation

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

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**

**inside of USA : 1 – 800 – 535 – 5053**

## Product characteristics of the InviMag® Universal Kit/ IG

The **InviMag® Universal Kit/ IG** is the ideal tool for an efficient and fully automated DNA extraction and purification e.g. from fresh or frozen whole blood samples or DNA/RNA from fresh or frozen plasma/serum using magnetic beads in combination with the InviGenius® robotic platform.

Starting Material	Yield	Time for preparation
200 µl fresh or frozen blood (EDTA / citrate stabilized, but <u>not</u> heparin), 200 µl fresh or frozen plasma/ serum 200 µl cell free body fluids (urine) 200 µl rinsed liquid from swabs 200 µl transport media (Surepath, Thinprep) 200 µl supernatant from stool suspension 200 µl from liquid sputum, BAL, tracheal secretes	depending on sample (storage and source)  <b>Note:</b> The added Carrier-RNA will account for most of the eluted viral nucleic acid(s). Quantitative (RT)-PCR is recommended for determination of the viral RNA or DNA yield	about 70 min per run (12 samples)

The DNA/RNA isolation process is based on the interaction of nucleic acids with silica coated magnetic particles in optimal buffer conditions.

The InviGenius® instrument will automatically perform all steps of sample and reagent distribution. The DNA/ RNA purification procedure is performed without any user intervention, except any sample pretreatment and the initial loading of the system. This allows safe handling of potentially infectious samples. Sample cross-contamination and reagent cross-over is effectively eliminated. The usage of unique bar codes for samples and reagents avoids unwanted transpositions.

The InviGenius® instrument uses magnetic rods to transport the DNA/RNA-binding magnetic particles through the various extraction phases: lysis-bind-wash-elute. Eliminating the direct liquid handling and increasing the automation level results in a fast, reliable and robust technique.

After a sample specific lysis using **Lysis Buffer HLT** and **Proteinase K**, optimal binding conditions are adjusted by the addition of **Binding Solution**. The DNA/ RNA binds to the added magnetic particles (**MAP Solution B**) and is separated from the solution by magnetic rods controlled by the InviGenius® system. Subsequent to three washing steps of the particle bound nucleic acids, the DNA/ RNA is finally eluted in **Elution Buffer M**.

Due to the high purity, the eluted total DNA/RNA is ready-to-use in a broad panel of downstream applications:

- PCR, real-time PCR
- Restriction Enzyme Digestion
- HLA Typing

For the isolation of DNA from a low number of samples STRATEC Molecular offers the Invisorb® Spin Universal Kit.

For high-throughput applications for up to 96 samples, STRATEC Molecular offers the HTS-Universal Kit line on InviSorb® or InviMag® basis on Hamilton and KingFisher instruments.

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



## Sampling and storage of starting material

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.

### **Blood**

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

### **Serum and plasma**

After collection and centrifugation, serum, plasma, 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 -20°C or -80°C. Frozen plasma or serum samples must not be thawed more than once. Multiple thawing and freezing before isolating any viral DNA/RNA should be avoided because it can lead to denaturation and precipitation of proteins, resulting in reduced viral titers and yields of viral nucleic acids. If cryoprecipitates are visible, 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 liquids from fresh prepared swabs as well as with dried swabs. The protocol has not been validated for isolation of RNA from swabs which are stored in storage buffers of other providers.

### **Cultivated bacteria or bacterial suspension(s)**

Bacteria have to be centrifuged after cultivation and resuspended at defined conditions. Best results are obtained with fresh material.

### **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 quickly lead to digestion and degradation of nucleic acids. These samples should be stored at -80°C.

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

## Principle and procedure

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

- lysis and protein digestion
- binding of the DNA/RNA to the magnetic beads
- washing the bead bound DNA/RNA and elimination of alcohol
- elution of DNA/RNA

After lysis, the nucleic acids bind to the magnetic beads whereas contaminations and enzyme inhibitors are efficiently removed during the following three washing steps.

### Lysis

Primary or pretreated samples are lysed at elevated temperatures in the presence of **Lysis Buffer HLT** and **Proteinase K/ Carrier-RNA** (PKC tube).

### Binding of the genomic DNA/RNA

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

### Removing residual contaminants

Contaminants are efficiently removed by washing steps while the DNA/RNA remains bound to the magnetic beads.

### Elution

The DNA/RNA is finally eluted in **Elution Buffer M**. The eluted DNA/RNA is ready-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 genomic DNA from Blood

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

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

Please keep in mind, that a small amount of Carrier-RNA in the eluate will elevate the real genomic DNA content.

## Yield and quality of pathogen DNA/ RNA

The amount of purified pathogen DNA/RNA in the **InviMag® Universal Kit/ IG** 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 is suitable for any molecular-diagnostic detection system.

Different amplification systems vary in efficiency depending on the total amount of nucleic acid present in the reaction. Viral eluates derived by this kit will contain Carrier-RNA, which will greatly exceed the amount of the isolated NA.

Yields of viral nucleic acids isolated from biological samples are usually low concentrated and therefore almost impossible to determine photometrically.\* Quantitative RT-PCR is recommended for determination of viral DNA or RNA yield.

\*. Keep in mind that the Carrier-RNA (5 µg per 200 µl sample) will account for most of the present RNA.

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

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

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

## Important notes

### Important points before starting a protocol

Immediately upon arrival of the product, inspect the kit and its components as well as the package for any apparent visible damages and correct quantities. If there are any unconformities, please notify STRATEC Molecular in writing with 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" (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 or merge components from kits.
- Avoid microbial contaminations of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend preparing samples under laminar air-flow.
- This kit should only be used by trained personnel.

## Preparing reagents and buffers

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

**Lysis Buffer HLT**, **MAP Solution B** and **Elution Buffer M** are ready-to-use.

### 4 x 12 DNA/RNA-extractions

Resuspend lyophilized material in **PKC Tube** by addition of 800 µl **RNase-free Water** (included) and mix thoroughly!

Add 80 ml of 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle labeled with Binding Solution

Add 84 ml of 96-100% ethanol to the bottle Wash Buffer II. Mix thoroughly and always keep the bottles firmly closed!

### 8 x 12 DNA/RNA-extractions

Resuspend lyophilized material in **PKC Tube** by addition of 800 µl **RNase-free Water** (included) and mix thoroughly!

Add 80 ml of 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle labeled with Binding Solution

Add 84 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

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). (See our webpage: [www.stratec.com](http://www.stratec.com))

- Measuring cylinder (250 ml)
- Pipette tips
- Disposable gloves
- ddH<sub>2</sub>O
- Vortex
- 96-100% ethanol
- 99.7 % Isopropanol\* (molecular biological grade)

\*) The **InviMag® Universal Kit/ IG** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth**.

### Possible suppliers for Isopropanol:

**Carl Roth**

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

**Applichem**

2-Propanol für die Molekularbiologie  
Order - No. A3928

**Sigma**

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

### Possible primary tubes, manufacturer, cat. no.

- Venosafe, 5.5 ml, Ref, VF-076SDK, Terumo
- Vacuette, 2 ml Ref, A110500I, Greiner bio-one
- Vacuette, 9 ml, Ref, 455036, Greiner bio-one
- BD Vacutainer, 2.7 ml, Ref, 363048
- BD Vacutainer, 6 ml, Ref, 367864
- BD Vacutainer, 10 ml, Ref, 367525
- BD Vacutainer 5.0 ml, Re
- Sarstedt Monovette, 8.5ml
- PS Tube Sarstedt 5 ml, Ref : 55.476
- Sarstedt Monovette 4.5 ml
- Sarstedt Monovette 7.5 ml
- Sarstedt Monovette 9.0 ml

### Possible supplier for lysozyme:

**Fa. Applichem**

Lysozyme, freeze dried  
Order No. A4972.0010

## Important indications

### 1. Minimum volume of samples in primary tubes

**Please provide at least 600 µl sample in the primary tubes!**

The procedure of the **InviMag® Universal Kit/ IG** is optimized for the isolation of DNA/RNA from up to 200 µl.

UUNI\_E100\_S200 with E100= 100 µl elution volume, S200= 200 µl sample volume

UUNI\_E200\_S200 with E200= 200 µl elution volume, S200= 200 µl sample volume

The dead-volume is dependent on sample tube. If the InviGenius® detects a low sample volume (<400 µl) it will aspirate 200 µl sample from the tube bottom and informs the user with the warning "Too low volume detected in sample container XX" This feature is a remark to check the sample tube after the run for a correct sample-transfer.

### 2. Sample volume smaller than 550 µl

Please adjust the sample volume to at least 600 µl. Samples lower than 550 µl may be flagged in the result report! (refer to point 1)

### 3. Elution volume

The final elution volume is 100 µl or 200 µl depending on the selected assay file

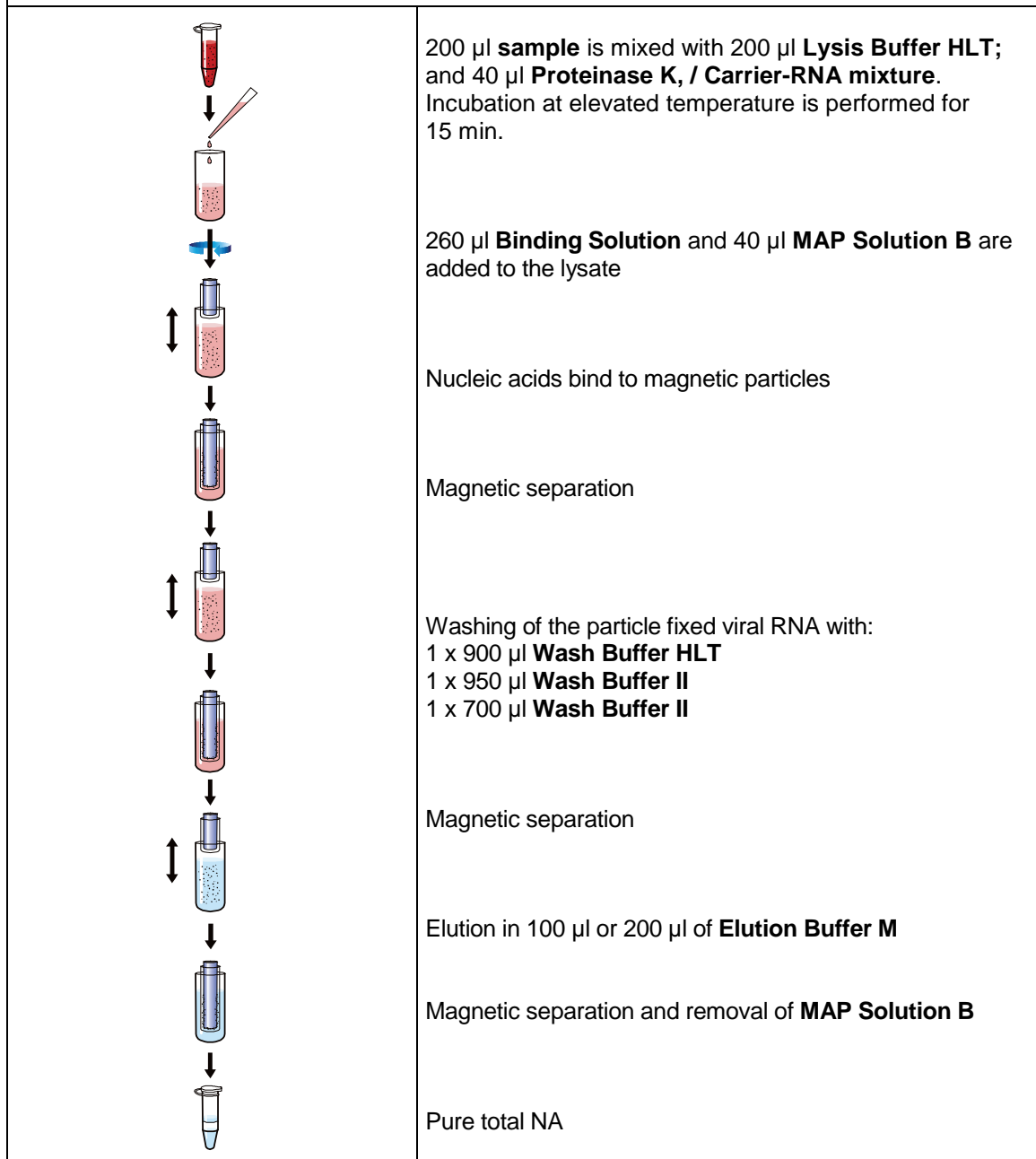
### 4. Processing of bacterial samples

The kit was validated with *Bacillus subtilis* spiked cell-free medium. To perform a quantitative extraction of bacterial DNA from gram positive bacteria, the addition of Lysozyme is required. Please add 5 µl of a 10 mg/mL Lysozyme-solution per 200 µl sample volume to the primary tube before starting the assay.

## Scheme of the InviMag® Universal Kit/ IG

**Add the primary tubes in the sample loading rack.**

**Add the Buffers in the Buffer loading rack.**



# Preparing the samples for processing on the InviGenius®-system

Please read the instructions carefully and conduct the prepared procedure.

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**Important Note:** *The protocol has been optimized for the isolation of gDNA and viral nucleic acids from up to 200 µl of liquid samples. To prevent possible distribution errors it is required to provide at least 550 µl of sample to ensure stable processing.*

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

These types of samples can be processed directly without any preparations. Please provide at least 550 µl sample or use RNase/DNase-free water or PBS to adjust to this volume.

Please keep in mind that the first step in the equipment is the premixing of samples. Therefore samples have to be at least “transferable”, which means no presence of clots and other solid materials that will lead to blocked pipette tips and prevent a normal workflow. We recommend checking samples for coagulation by mixing several times before usage on the instrument.

## 2. Extraction of NA from rinsed liquid from swab samples

a) the sample will be used for cultivation

Cut off the relevant part of the swab and transfer it into an RNase/DNase-free 2 ml tube. Add 550 µl physiological saline solutions to the swab and vortex intensely for 2-3 min. Then incubate for 10 min at RT. Use an aliquot for further cultivation. Transfer 500 µl of the rinsed liquid into a primary tube. If bacterial DNA is processed 50 µl Lysozyme (not provided, stock solution 10 mg/ml) has to be added.

**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 500 µl RNase-free water to the swab and vortex intensely for 3 min. Optional, incubate for 3 min at 95°C. Transfer the rinsed liquid into a primary tube. If bacterial DNA is processed 50 µl Lysozyme (not provided, stock solution 10 mg/ml) has to be added.

## 3. Extraction of NA from sputum

Transfer 300 µl from the sputum sample into an RNase/DNase-free tube and add 300 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 the mixture into the primary tube. If bacterial DNA is processed 60 µl Lysozyme (not provided, stock solution 10 mg/ml) has to be added.

## 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 500 µl RNase-free water and transfer the sample into a primary tube. Add 50 µl Lysozyme (not provided, stock solution 10 mg/ml) to the tube.

**Viscous sample:** Transfer 1 ml of tracheal secretate or BAL into a RNase/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.

Resuspend the bacterial pellet in 500 µl distilled water or RNase-free water and transfer it into a primary tube. Add 50 µl Lysozyme (not provided, stock solution 10 mg/ml) to the tube.

#### **5. Extraction of viral NA from supernatant of stool suspension**

Transfer 100 µl stool sample into a 2 ml tube and dilute the sample 1:10 with 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 550 µl 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 500 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 the bacteria containing supernatant into the cavity of a primary tube (prevent the aspiration of swimming particles) and add 50 µl Lysozyme (not provided, stock solution 10 mg/ml) to the sample.

#### **Prevention of cross-contamination**

To comply with the demanding guidelines of *in-vitro* diagnostics we programmed the InviGenius® to route the pipettor in such a way that possible contamination-risks are minimized. However we recommend to apply the supplied well-strips and sealing foils beforehand (and afterwards on the used wells) on the unused wells of the Incubation Plate A and the Working-Plate A. Be careful not to seal any required lanes of the working plate (four first free lanes).



## Preparing of the internal control for the InviGenius<sup>®</sup>-system

Please read the instructions carefully and conduct the prepared procedure.

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### Using an internal control (IC)

Using the InviGenius Kit in combination with a commercially available amplification system 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 Carrier RNA stock solution (or with Carrier RNA - Proteinase K - stock solution) in one mixture. For each sample the machine transfers a volume of 40 µl of the stock PKC solution to the lysis mix.

The PKC vials contain 800 µl stock solution, so the internal control for 20 samples must be added directly to the PKC tube.

### Example – Calculation:

Per Extraction 4.5 µl of the DNA control would be needed:

$$4.5 \mu\text{l} / \text{RXN} \times 20 \text{ RXN} = 90 \mu\text{l}$$

→ PKC stock solution has to be made by adding 800 µl – 90 µl = 710 µl RNase-free water. Then add 90 µl control DNA followed by a mixing step.

### 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 can alternatively be added to the sample shortly before beginning sample preparation. But consider that a bigger amount of internal control is necessary when using bigger volumes of primary sample tubes. The minimal required sample volume in the sample tube has to be at least 550 µl.

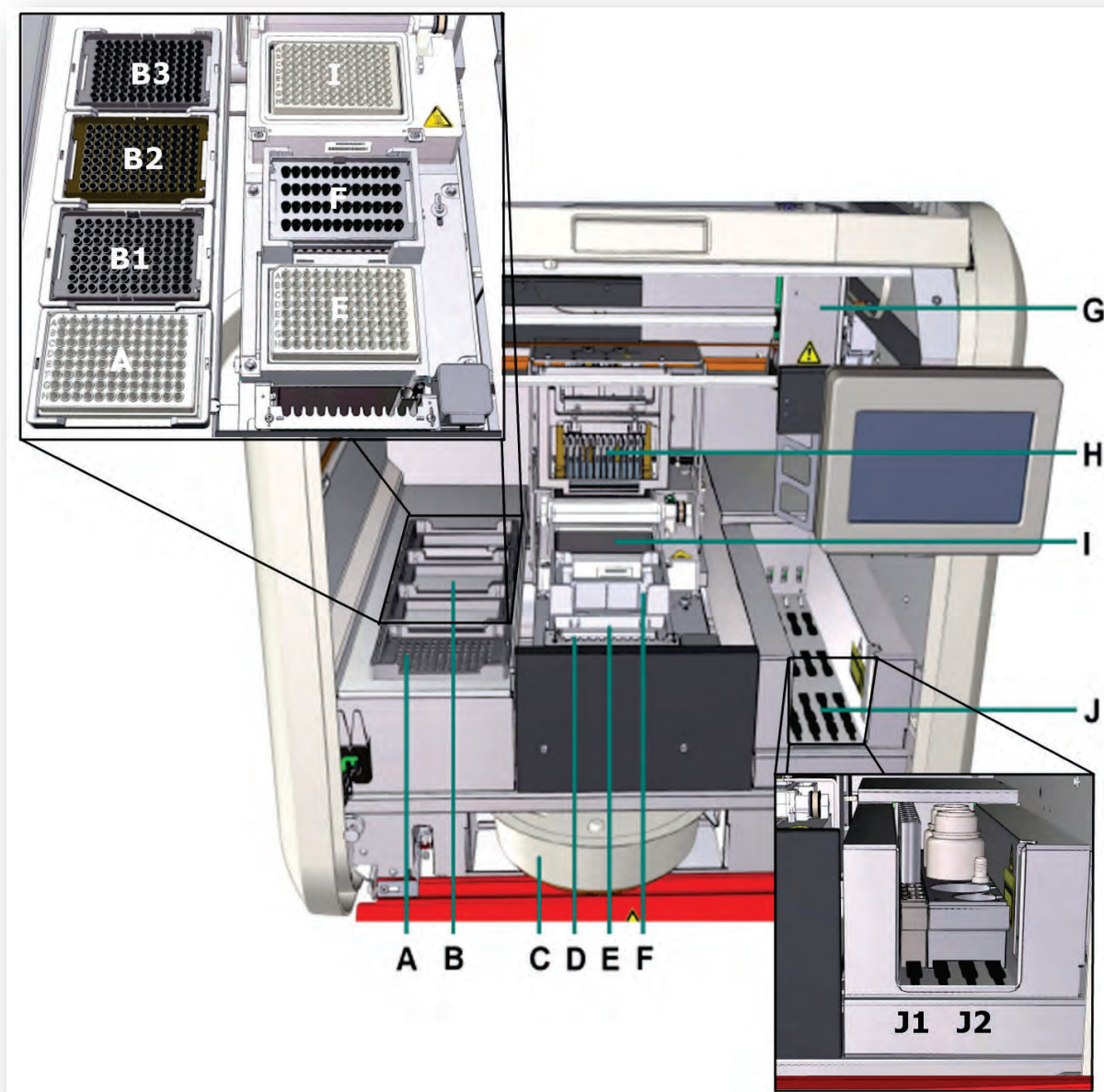
If the internal control (IC) is naked/unprotected DNA or RNA and it is unstable in plasma, serum, CSF, urine, respiratory samples, whole blood, stool, transport media, or on dried swabs, the internal control must not be added directly to the samples.

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

### Carrier-RNA

Carrier-RNA serves two purposes: It enhances the binding of nucleic acids to the beads, especially if there are only very few target molecules in the sample. Furthermore, the addition of large amounts of Carrier-RNA reduces the chance of nucleic acid degradation.

## General overview of the InviGenius® system



**Figure 1: Frontal view of the InviGenius® system**

Figure 1 shows the plate positions **A** (elution position), **E** (working position) and **I** (incubation position). Disposable tips are placed on position **B1-B3** and disposable sheaths on **F**. The waste tray **C** is located on the lower side of the InviGenius® system behind the red flap. The waste shaft **D** is completely stainless steel and easy to remove for autoclaving.

The loading bay is divided into sample loading bay (**J1**) and reagent loading bay (**J2**). The magnetic separator head (MSH) **H** is located on top of the incubator **I** and can reach all necessary positions. The single head pipettor **G** starting positions are in the right front of the machine. All movable parts only work when the InviGenius® machine is closed and locked.

## Preparing and loading of the InviGenius® system

### Preparing the reagents

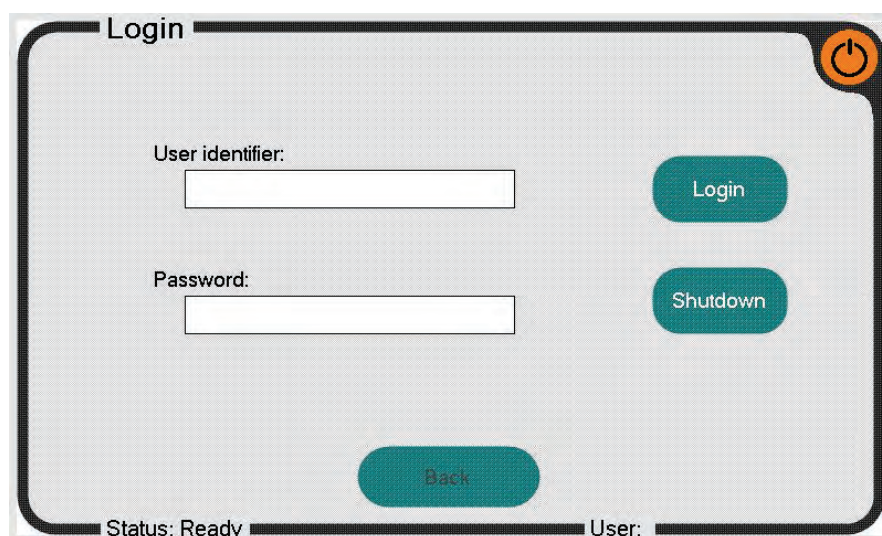
Before you start a new kit, add ethanol to Wash Buffer II.

Before starting a new run dissolve one vial of PKC with 800 µl RNase-free water. Refer to page 16 if an internal control is used.

### Preparing the system

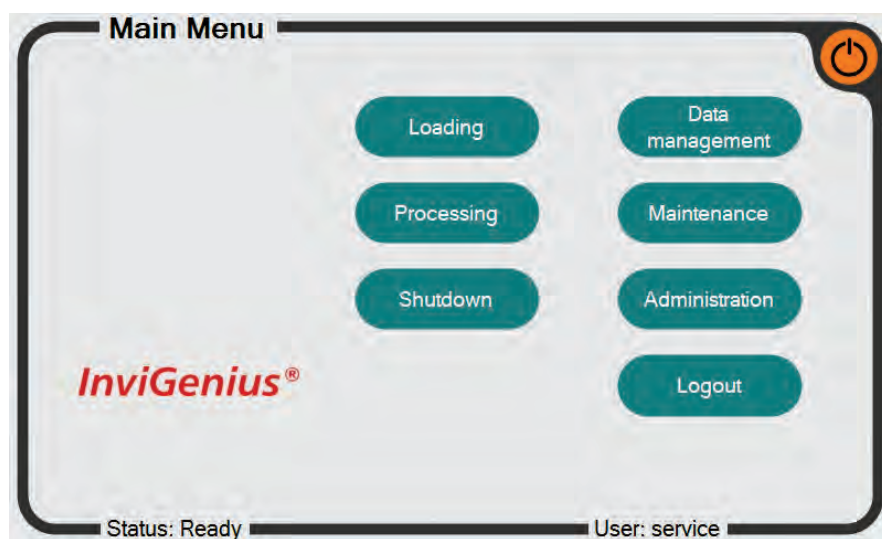
Switch on the InviGenius® system using the power switch located on the right side of the back part of the instrument. The InviGenius® software will be automatically loaded after the system has booted up. Please keep the door of the InviGenius® system closed during system initialization.

After initialization of the InviGenius® system a login screen appears (Figure 2). Log-in with the provided user name and password.



**Figure 2:** Login screen of the InviGenius® software

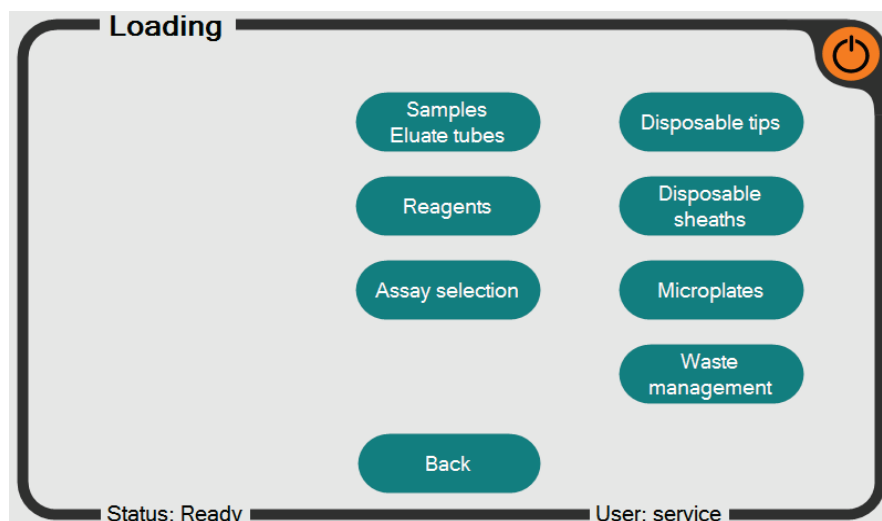
After logging in the main screen of the InviGenius® software appears (Figure 3). Select “Loading” to start with the loading procedure of the system and prepare. Go directly to “Processing” to define and run an assay if the system has been already loaded.



**Figure 3:** Main menu of the InviGenius® software

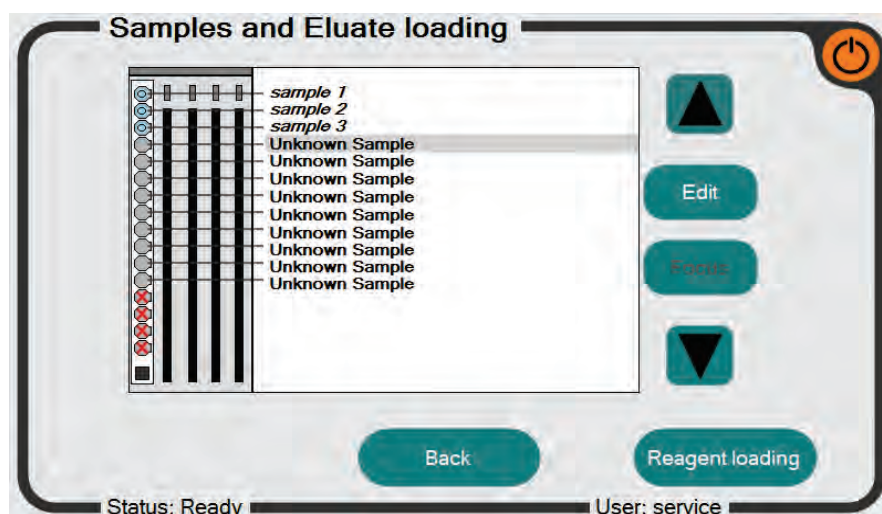
## Sample Loading

After selecting “Loading” the sample loading screen appears.



**Figure 4:** Loading screen of the InviGenius® software

Select “Samples” to proceed with the sample-loading-screen.



**Figure 5:** “Sample-loading” screen of the InviGenius® software

Please add the samples to the rack and decap all tubes before transferring them into the rack. If available, primary tubes should be used directly as sample tubes. If the samples are not provided in primary tubes, please prepare the sample rack with primary tubes that are prefilled with samples from which the nucleic acids shall be extracted. Sample tubes are not provided with the kit but can be ordered at e.g. Sarstedt (order no. 55.476, 5 ml tubes, 75x12 mm, PS) or refer to recommendation at page 11, chapter “Reagents and equipment to be supplied by user”.

For each reaction a sample volume of 200 µl is processed. However, it is recommended that the total sample volume filled in the sample tubes should be at least 550 µl to ensure stable processing. Please take care, that only the first 12 positions of the sample rack will be processed due to the limited number of wells per row of the plastics. For correct identification of the sample tubes the unique bar codes must face to the bar code scanner located at the right side of the loading bay.

After inserting the sample rack in the very left lane of the loading bay, an updated screen will show the identifiers read from the sample bar codes (Figure 5). In case of unsuccessful sample identification, remove the rack, check the bar code orientation, and reinsert the rack slowly. It is

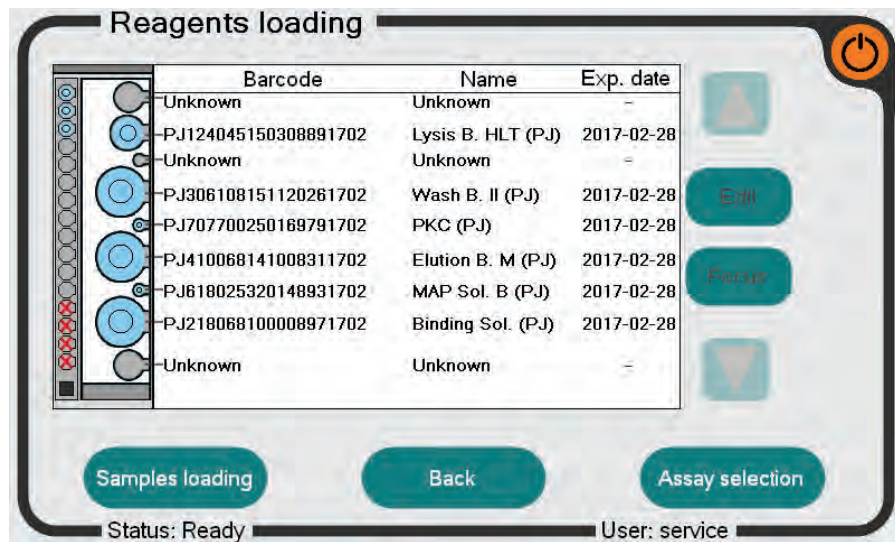
possible to rename samples by selecting the corresponding sample by using the arrow fields, followed by pushing the “Edit” button.

After a certain time (app. 5 min) the bar code scanner is deactivated. In that case the user has to restart the scanner with the “Focus” button if the loading procedure was not finished.

After successful loading of the samples proceed with reagent loading by selecting “Reagent loading” on the bottom right hand side of this screen.

## Reagent Loading

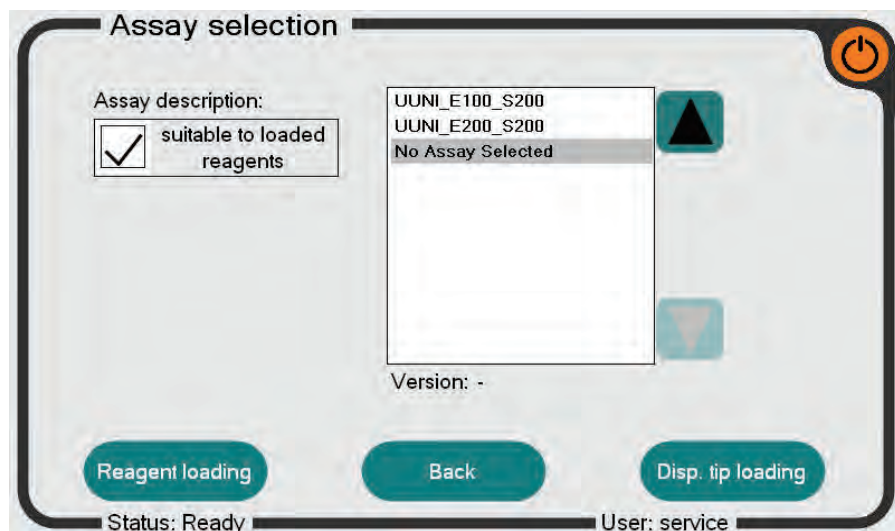
The reagent loading process is analogous to the sample loading procedure.



**Figure 6:** “Reagent-loading” screen of the InviGenius® software

Insert all provided reagents into the reagent rack of the InviGenius® system. Verify that the bar code labels face to the right side of the loading bay and decap the bottles and tubes. The order of the inserted reagents is not crucial because the type and position of a reagent is identified by the unique bar code. However, the possible loading positions are limited by the size of the bottles. After rack insertion the loading status of the reagents will be shown. In case of unsuccessful reagent allocation, remove the rack, check the bar code orientation and try again slowly.

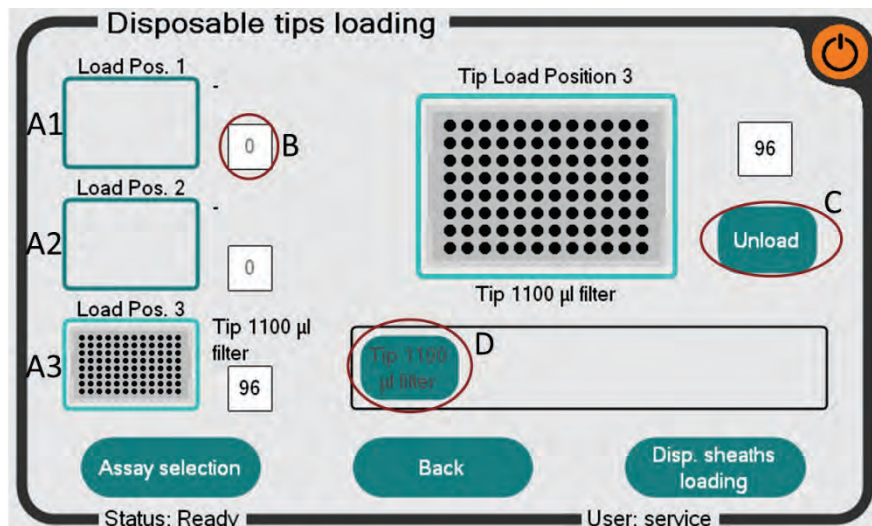
## Assay Selection



**Figure 7:** “Assay Selection” screen of the InviGenius® software

Select the appropriate assay and proceed with disposable tip loading. After assay selection the system will check the availability of the buffers, shelf live, and buffer volume. If reagents are missing that are required for the run, no assay will be visible or selectable.

## Disposable Tip Loading



**Figure 8**, Disposable tip loading screen

There are three tip rack positions on the InviGenius® system (Fig. 8, A1-A3). Remaining tip-numbers are shown in field (B). Tip-numbers can be changed by pressing the number-field directly.

Empty tip-racks can be unloaded and reloaded by:

- 1.) Pressing the Loading-Position directly (The software will focus the corresponding loading position on the main screen)
- 2.) Pressing the Unload-Button (C)
- 3.) The loading-position can be refilled with a new tip-rack by pressing on the corresponding tip-rack (D)

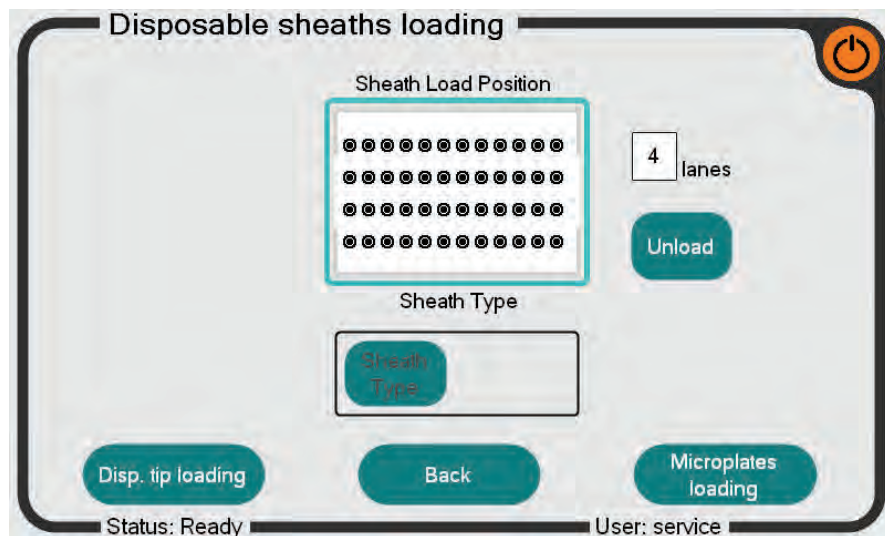
Each position can be filled either with 50 µl or 1100 µl filter tips. However, both Universal assays operate with 1100 µl filtered tips only.

All protocols should be used in combination with filter tips to ensure efficient prevention of sample or reagent cross-contaminations. STRATEC Molecular will give no guarantee or responsibility if contaminations occur due to the use of non-filtered tips.

**Note:** Disposable tips are not supplied within the kit. We recommend the use of validated conductive tips, which can be ordered at STRATEC Molecular. STRATEC Molecular offers 50 µl conductive tips (10x 96 pieces, order no. 5011120100) and 1100 µl conductive tips (10x 96 pieces, order no. 5011120200). Be sure that conductive tips are used otherwise the tip detection unit, installed in the pipetting unit, will reject the tips and no run is possible.

## Disposable Sheaths Loading

The sheaths are used as protection devices for the magnetic rods. They are automatically picked up during the run.



**Figure 9**, Disposable sheaths loading screen

The loading procedure of the disposable sheaths works analogous to the disposable tip loading screen. For a run, always 12 disposable sheaths (one row in the sheaths rack) are used, regardless of the processed sample numbers. This is done, to assure that the rods are always protected against contaminations.

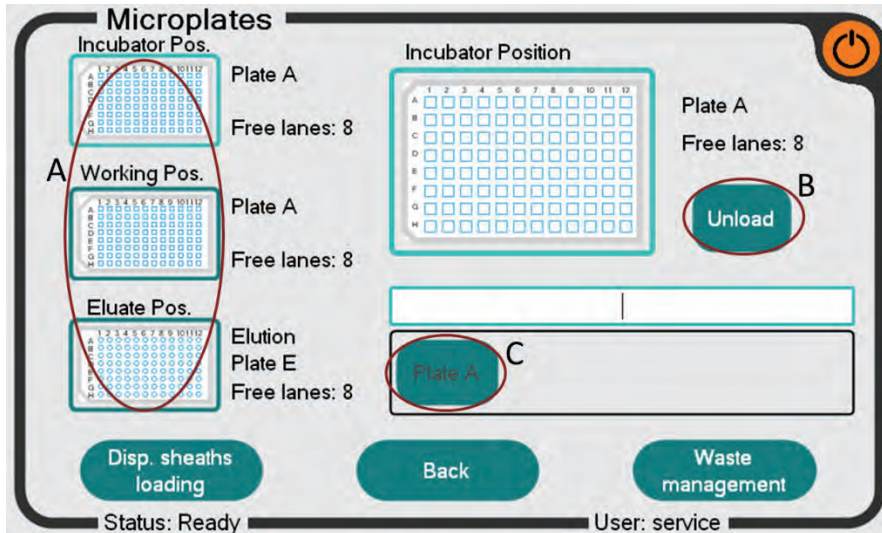
In general, the number of sheaths supplied within the kit is sufficient for the amount of runs imprinted on the kit package. If you are lacking sheaths, they can be ordered separately at STRATEC Molecular (100 pieces bulk, order no. 5011120300 or 10 x 48 pieces, order no. 5011120400).

Comparable to the disposable tips loading it is possible to define the number of rows left in the tip rack by pressing on the displayed number area. Make sure that the disposable sheaths are loaded (and displayed) consistent to the manually loaded sheaths in the rack to ensure correct sheaths pick up. Do not remove single disposable sheaths within a row of the sheaths rack if less than 12 samples are processed within one run. There is a sheaths detection sensor installed in the device. If less than 12 sheaths are picked up by the instrument a warning will be displayed and all picked up sheath are discarded into the waste container before the next row of sheaths are picked up for verification.

To avoid unwanted contaminations, we strongly recommend to not wash/reuse any disposed sheaths!

## Plate Loading

Analogous to the previous loading screens, the incubation, working and elution plate are loaded within the plate loading screen (Figure 10).



**Figure 10**, Plate loading screen

In general, the Incubation Plate A and Working Plate A (identical) are used at the incubator and working position whereas at the eluate position an Elution Plate E is used.

Used plates can be unloaded and reloaded by:

- 1.) Pressing the plate position directly (A). The software will focus at the plate position on the main screen.
- 2.) Pressing the “Unload” button (B)
- 3.) The plate can be reloaded by pressing at the offered plate in (C).

For a successful run the InviGenius® needs one free lane in the incubator plate, four free lanes in the working plate and one free lane in the eluate plate.

Please make sure that the depicted lanes shown on the monitor are consistent with the real lanes in the corresponding positions.

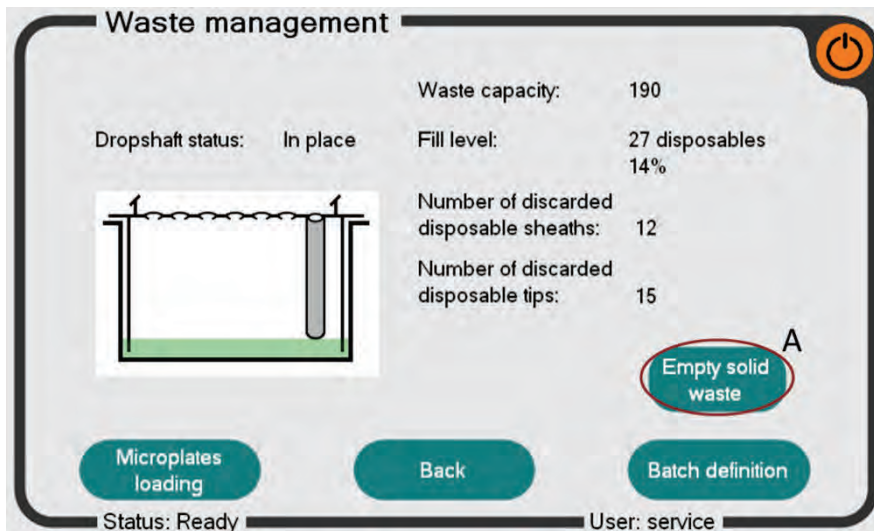
To avoid contaminations, we strongly recommend to not wash/reuse disposed plates!



## Waste management

Please make sure that the waste tray capacity is sufficient for the planned assay. If the capacity is not sufficient, empty the solid waste.

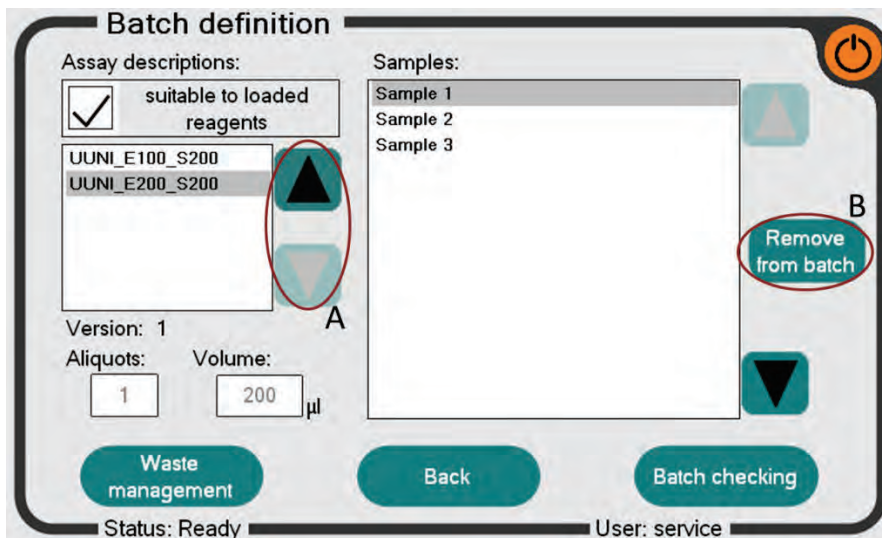
Note: The waste is potential infectious.



**Figure 11;** Waste management screen

If the waste tray was renewed, please click on the “Empty solid waste” button (A).

## Batch definition



**Figure 12;** Batch definition-screen

Please select the desired assay and recheck the allocated samples that should be processed in this run. It is possible to switch between the offered assays by using the two arrow buttons (A).

By default, all loaded samples are selected to be processed in this run. If samples have to be excluded from the batch, exclude them by selecting the corresponding sample followed by a click on the “Remove from batch” button (B).

## Batch checking

This screen shows a summary of all verified positions such as disposables, samples and reagents in one informational screen. Please ensure that all required components are loaded properly. In case of any error, the corresponding field will be highlighted in red color. In case that no error is displayed, proceed by pressing the button “Batch processing”.

To solve any error, directly click on the red highlighted field and follow the instructions printed on the instrument screen.

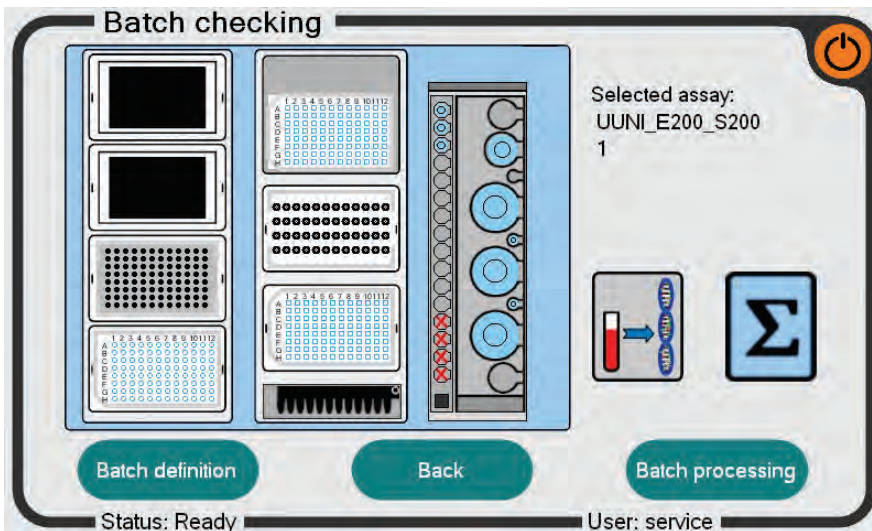


Figure 13; Batch checking screen

## Batch processing

After closing the system-door, the assay can be started by pressing the “Start”-Button (A). The door will be locked during the run and the system will start with sample processing. The door will only be unlocked after a run has been successfully finished or if an error occurs that requires user interaction. Do not try to force open the door during a run. This will cause an abort of the run!

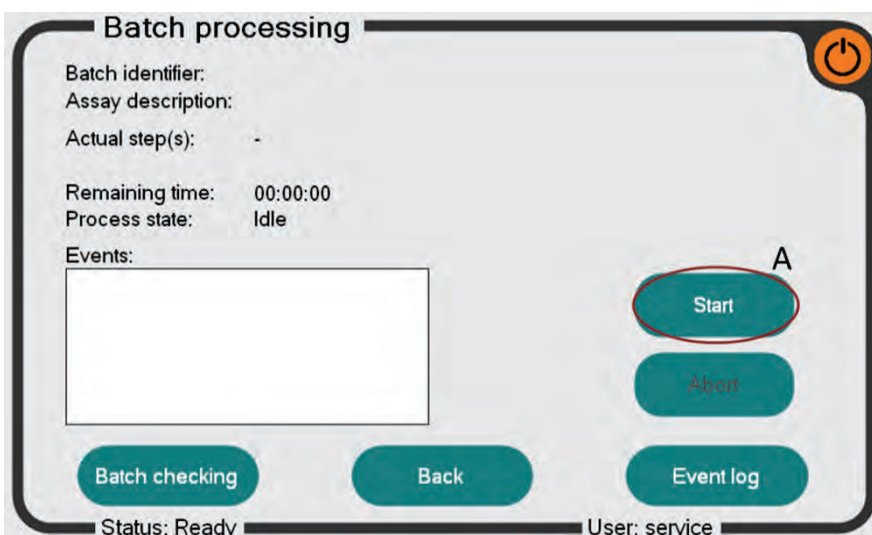


Figure 14; Batch processing screen

At the end of the process, the nucleic acid containing eluates are located in the appropriate eluate position and can be used for any further downstream application.

**Note:** *The complete process will take approximately 70 minutes.*

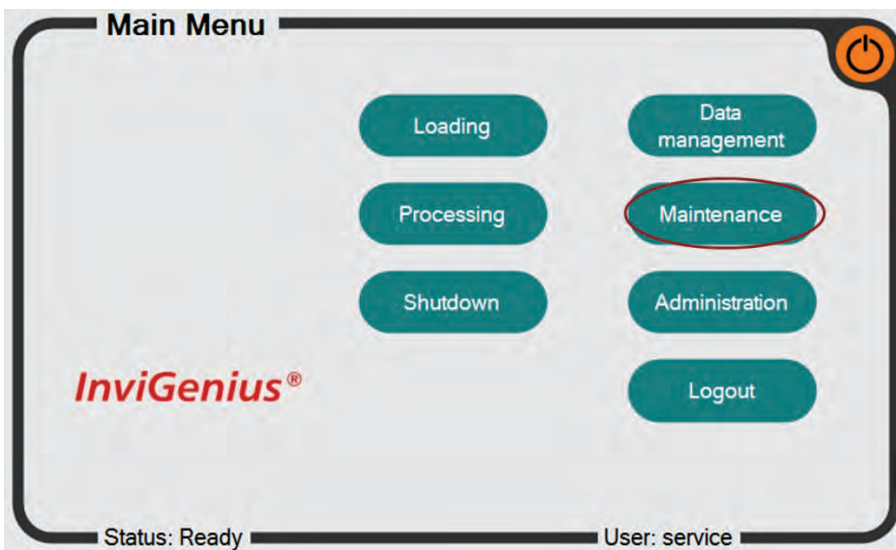
## After a run

After a run is completed and no additional run shall be started, unload all plates and reagents and store them according to GLP guidelines. Please keep in mind, that the plates could contain infectious material.

As with all medical/clinical and diagnostically equipment, all waste (liquids, tips, sheaths and plates) should be treated as potentially dangerous bio-hazard waste.

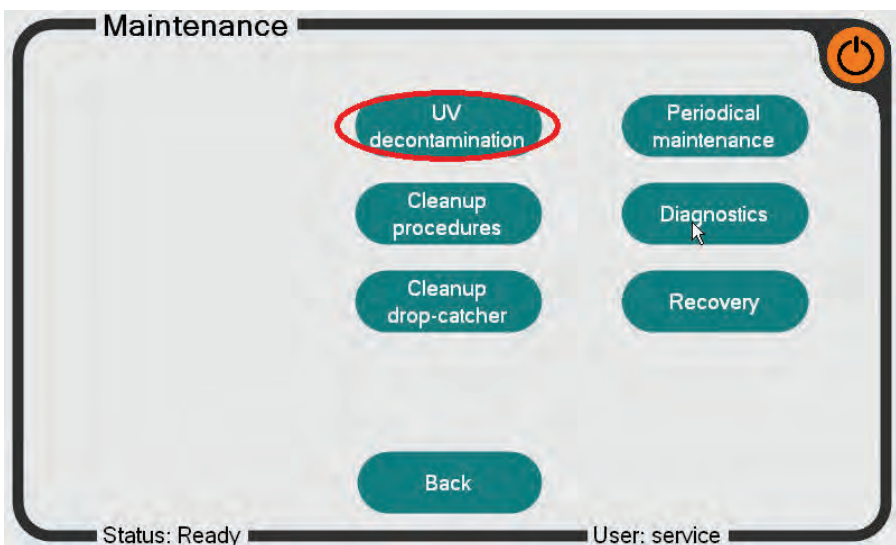
## Daily maintenance (UV decontamination)

The InviGenius® system is equipped with an internal UV lamp (254 nm wavelength) that should be used daily either at the end of the working day or in the morning before a run is started. The suggested decontamination time is about 20 min. To start the UV decontamination go to the main menu of the InviGenius software and select “Maintenance”.



**Figure 15:** Main screen of the InviGenius® software

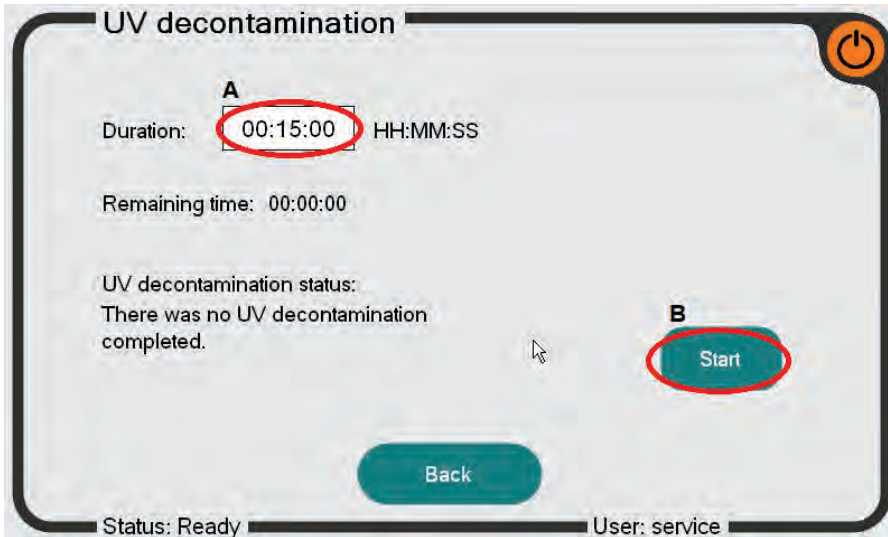
When the sub item “Maintenance” is opened, select “UV decontamination”



**Figure 16:** Maintenance screen of the InviGenius® software

In the UV decontamination menu adjust the exposure time (A) and finally press the “Start” button (B). During the decontamination process the instrument door will be locked to prevent any UV radiation release in the lab.

**Warning: UV radiation is harmful. It causes serious burns of the skin and leads to irreparable damage of the eyes and skin. Ensure that no lab personnel is submitted to direct UV light. Do not try to force open the instrument door during the decontamination process.**



**Figure 17:** UV decontamination screen

When the decontamination is finished, go back to the main menu by using the “Back” button. The device is now decontaminated and can be either switched off or used for sample processing. We recommend decontaminating the instrument daily.

## 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 gDNA is necessary to ensure its functionality in various downstream applications. Damaged DNA/RNA could perform poorly in applications such as Southern blotting, long-template PCR, and construction of cosmid libraries.

#### 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 or at -20°C. Please remark that storage at -20°C can cause shearing of DNA if the sample is exposed to repeated freeze-thaw cycles. Viral RNA should be stored for long-term storage at -80°C.

## Troubleshooting

Problem	Probable cause	Comments and suggestions
pipetting distribution errors	pipetting of <b>PKC</b> failed	ensure that the lyophilized <b>PKC</b> is diluted with the appropriate volume of water before usage
	samples transfer failed / incomplete	the sample tube must contain at least 500 µl sample to avoid a low liquid warning
	reagent / buffer transfer failed / incomplete	ensure that the supplied <b>Wash Buffer II / Binding Solution</b> are filled up properly with either ethanol or isopropanol  do not reuse bottles more often than described in Tab.1 because they will be rejected by the system
low concentration of extracted RNA /DNA	sample components settled	in case of large sample volumes (>>1 ml) carefully premix the sample tube before inserting it into the sample rack
	No / too much ethanol added to <b>Wash Buffer II</b>	ensure that the <b>Wash Buffer II</b> have been filled up properly with ethanol as indicated in Tab. 1
degraded RNA / DNA	incorrect storage of starting material	ensure that the storage of starting material is correct  avoid multiple freezing and thawing cycles of the material
	old material	ensure that the starting material is fresh or stored at appropriate conditions (for long-term storage freeze at -20°C)!  avoid multiple thawing and freezing cycles of the material  old material may contain degraded DNA
no assay selectable	combination of reagents from different kits / missing required reagent	ensure that only and all reagents belonging to one kit type are used. a combination of reagents belonging to different kit types is not supported
eluted nucleic acids are brownish colored	Residual magnetic particles are left in eluate	centrifuge the eluate at full speed for 1 min and transfer supernatant to a new tube

## Ordering information

Product	Package size	Catalogue No.
InviMag® Universal Kit/ IG	8 x 12 preps	2450120100

Related Products	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
InviMag® Universal Kit / STARlet	4 x 96 preparations	7450330300
InviMag® Universal Kit / STARlet	24 x 96 preparations	7450330400
InviMag® Universal Kit /KF96	1 x 96 preparations	7450300200
InviMag® Universal Kit /KF96	5 x 96 preparations	7450300300
Invisorb® Spin Universal Kit	50 preparations	1050100200
Invisorb® Spin Universal Kit	250 preparations	1050100300

### InviGenius® and consumables

InviGenius®	1 unit	5011100000
Starting Box I/ IG	1 box	2400110100
Sheath Box Conductive filter tips, 1 ml; 2 x 2 rack/ pack (384 pieces) 5 Waste Trays 120 sample tubes		
Sheath Bundle	10 x 48 pieces	5011100300
Sheaths	1000 pieces	5011100200
Conductive filter tips, 1 ml	10 x 96 pieces	5011100400
Waste tray/ IG	25 pieces	5011100100

### Possible suppliers for Isopropanol

#### Carl Roth

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

#### Applichem

2-Propanol für die Molekularbiologie  
Order no. A3928

#### Sigma

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



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