INVITEK
Molecular

USER MANUAL

InviMag® Free Circulating DNA Kit/ IG

for fully automated purification of cell-free DNA from plasma, serum or cell-free body fluids
with magnetic beads on the InviGenius® PLUS

CE IVD REF 2439320400

Invitek Molecular GmbH · D-13125 Berlin

Instruction

InviMag® Free Circulating DNA Kit/ IG

The **InviMag® Free Circulating DNA Kit/ IG** is designed for fully automated extraction and purification of cell-free circulating DNA from 4 ml of serum or plasma samples. Up to 12 samples can be processed using the InviGenius® PLUS robotic platform.

The **InviGenius® PLUS** is a compact walk-away DNA/RNA extraction platform with full in-process control, including the following modules such as pipettor, heat incubator, barcode reader, magnetic separation head, integrated PC and touch screen, barcode labelled sample rack for primary tubes and a barcode labelled reagent rack, which helps to deliver premium quality nucleic acid for routine laboratories. The workstation eliminates human errors, standardizes the extraction process, and offers an integrated solution for data storage, backup and archiving. The unique bar codes for samples and reagents avoid unwanted transpositions.

The kit is not validated for the isolation of genomic DNA from whole blood or blood stains.

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

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

Contents

Kit contents of InviMag® Free Circulating DNA Kit/ IG

Component	8 x 12 preps	
Catalog No	2439320400	
Lysis Buffer HLT	8 x 90 ml	12 samples (in max. 3 runs)
Proteinase S	8 x 3000 µl	12 samples (in max. 2 runs)
MAP Solution B/ IG	8 x 3000 µl	12 samples (in max. 2 runs)
Binding Buffer RV	8 x 60 ml (final volume 8 x 100 ml)	12 samples (in max. 3 runs)
Carrier RNA	4 tubes	
Carrier RNA Solution	4 empty tubes (final volume 4 x 1800 µl)	24 samples (in max. 4 runs)
RNAse free Water	15 ml	
Wash Buffer R2	2 x 25 ml (final volume 2 x 125 ml)	48 samples (in max. 12 runs)
Ethanol	2 empty bottles (final volume 2 x 50 ml)	48 samples (in max. 12 runs)
Elution Buffer M	2 x 30 ml	36 samples (in max. 12 runs)
Incubation Plate D	2 x 4	1 run per plate
Working Plate B	2 x 4	1 run per plate
Elution Plate E	1	8 runs per plate
Sheath Box	1	
Microtube Cap	8	
Initial steps	Add 100 ml of 96-100 % Ethanol to each bottle **Wash Buffer R2** and mix thoroughly Fill 50 ml of 96-100 % Ethanol into the empty bottle Ethanol If there are any precipitates within the **Binding Buffer RV** solve these precipitates by warming carefully (up to 30°C). Add 40 ml 99.7% Isopropanol (molecular biologic grade) to the bottle **Binding Buffer RV** directly before the run Resuspend each **Carrier RNA Tube** in 1800 µl RNase free Water, solve the Carrier by at least 30 s vortexing (check for precipitates) and **transfer the fluid in a provided empty tube,** which is signed with **"Carrier RNA Solution".** Each tube with **"Carrier RNA solution"** is enough for two runs with each 12 samples.	

Symbols

 Manufacturer

 Lot number

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

 Catalogue number

 Expiry date

 Consult operating instructions

 Temperature limitation

 Do not reuse

 Humidity limitation

Storage

All buffers and kit contents of the **InviMag® Free Circulating DNA Kit/ IG**, except **dissolved Carrier RNA Solution** 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).
Carrier RNA: Dissolved Carrier RNA must be stored at -20°C. Therefore, the dissolved mix is stable as indicated on the kit package.
Wash Buffer charged with ethanol should be appropriately sealed and stored at room temperature.
Binding Buffer RV should be charged with isopropanol directly before the run. The buffer should be appropriately sealed and stored at room temperature. If there are any precipitates within the Binding Buffer RV solve these precipitates by warming carefully (up to 30°C).

Quality control and product warranty

Invitek Molecular warrants the correct function of the **InviMag® Free Circulating DNA 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, Invitek Molecular will check the lot and if Invitek Molecular investigates a problem in the lot, the product will be replaced free of charge.

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

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

If you have any questions or problems regarding any aspects of **InviMag® Free Circulating DNA Kit/ IG** or other Invitek Molecular products, please do not hesitate to contact us. A copy of Invitek Molecular's terms and conditions can be obtained upon request or are presented at the Invitek Molecular webpage.

For technical support or further information, please contact:
Email: techsupport@invitek-molecular.com or contact your local distributor.

Intended use

Most of the DNA in the body is located within cells, but a small amount of nucleic acids can also be found circulating freely in the blood. These DNA molecules are thought to come from dying cells that release their contents into the blood as they break down. The term "Circulating Nucleic Acids" refers to segments of DNA or RNA found in human cell free blood derivate like serum or preferably plasma.

Circulating DNA offers a non-invasive blood based approach to a wide range in diagnostics of clinical disorders. Analysis of this DNA allows the basic information necessary not only for use in predictive medicine but also for direct use in acute medicine. One of these diagnostic tests is the prenatal detection of fetal DNA for chromosomal aberrations. Another diagnostic application is the non-invasive monitoring of cancer diseases.

For a detailed review of the significance of circulating nucleic acids in diagnostic approaches we recommend the book “Circulating Nucleic Acids in Early Diagnosis, Prognosis and Treatment Monitoring” (Peter P. Gahan, 2015).

The **InviMag® Free Circulating DNA Kit/ IG** is designed for the fully automated extraction and purification of free circulating DNA from serum or plasma. Up to 12 samples can be processed using a magnetic beads system and the InviGenius® PLUS robotic platform.

It is advised to provide at least 4500 µl sample per tube to prevent pipetting distribution errors due to the liquid level detection (LLD) process (more if the tube diameter is bigger than 12 mm). If the sample volume is smaller please add physiological saline solution (0.90% w/v) or PBS up to 4500 µl or use the assays without sample transfer (MT, it means manual transfer). In these assays, add exactly 4 ml of the samples in the Incubation Plate D. This processing saves sample volume and conserves nucleic acids. The final processed sample volume is 4000 µl. The nucleic acid isolation protocol is suitable for routinely walk-away automated preparation of free circulating DNA from fresh or frozen serum or plasma. For reproducible and high yields, appropriate sample storage is essential.

Common collection tubes (not provided) and anticoagulants (EDTA and citrate, *but not heparin*) can be used to assemble a set of samples. All utilities (reagents and plastics), except Conductive filter tips (must be ordered separately from Invitek Molecular GmbH), required for preparation of DNA are provided by the **InviMag® Free Circulating DNA Kit/ IG.**

THE PRODUCT IS ITDENDED 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 followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

The kit complies with EU Directive 98/79/EC on in vitro medical devices. However, it is not for in-vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Product use limitation

The kit is validated for the isolation of DNA from serum or plasma. Related applications will need a separate validation. The included chemicals are only useable once. Differing of starting material 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 Invitek Molecular product for any particular use. Invitek Molecular does not provide validations of performance characteristics of the product with respect to specific applications.

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

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

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

The chemicals and the 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.invitek-molecular.com for each Invitek Molecular product and its components. If buffer bottles are damaged or leaking, WEAR GLOVES, AND PROTECTIVE GOGGLES when discarding the bottles in order to avoid any injuries.
Invitek Molecular has not tested the liquid waste generated by the InviMag® Free Circulating DNA Kit/ IG procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste has be considered infectious and be handled and discarded according to local safety regulations. European Community risk and safety phrases for the components of the InviMag® Free Circulating DNA Kit/ IG to which they apply, are listed below as follows:

Lysis Buffer HLT

Warning

H302-H315-H319-P280-P305+P351+P338

Proteinase S

Danger

H317-H318-P280-P305+P351+P338

Binding Buffer RV

Warning

H302-H312-H319-H332-H412 -P280, P305+P351+P338 EUH032

H302: Harmful if swallowed.
H312: Harmful in contact with skin.
H315: Causes skin irritation.
H317: May cause an allergic skin reaction.
H318: Causes serious eye damage.
H319: Causes serious eye irritation.
H332: Harmful if inhaled.
H412: Harmful to aquatic life with long lasting effects.
P280: Wear protective gloves/protective clothing/eye protection/face protection
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes.
Remove contact lenses, if present and easy to do. Continue rinsing.
EUH032: Contact with acids liberates very toxic gas.

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

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

Product characteristics of the InviMag® Free Circulating DNA Kit/ IG

The **InviMag® Free Circulating DNA Kit/ IG** is an ideal tool for efficient and fully automated cell-free circulating DNA extraction and purification from fresh or frozen samples using magnetic beads in combination with the InviGenius® PLUS system.

Starting material	Yield	Time for preparation
up to 4000 µl serum or plasma. To prevent pipetting errors due to dead-volume, please provide 4500 µl in the sample tube or add physiological saline solution (0.90% w/v) or PBS up to 4500 µl in the sample tube. Add 4000 µl directly in the Incubation Plate D when using assays without sample transfer.	depends on the sample (source and storage) ***Note:*** The added Carrier RNA will account for most of the eluted DNA. Quantitative RT-PCR is recommended for determination of the DNA yield.	ca. 160 min for 12 samples without sample transfer

The DNA isolation process is based on the interaction of nucleic acids with silica coated magnetic particles at adapted buffer conditions. The InviGenius® PLUS instrument will automatically perform all steps of sample and reagent distribution. The DNA purification procedure is performed without any user intervention, except the initial loading of the system, thus allowing safe handling of potentially infectious samples. Sample cross-contamination and reagent cross-over is effectively eliminated by the automated purification process. The use of unique bar codes for samples and reagents avoids unwanted transpositions.

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

The Kit is optimized for using batch sizes of 12 samples. If using batches of lower than 12 samples some of the plastics and even some buffers may be insufficient for processing 96 samples, the instrument will recognize this and give respective warnings.

Due to the high purity, the eluted circulating DNA is ready-to-use in a broad panel of downstream applications such as:

- real-time PCR* (quantitative RT-PCR, like TaqMan® und LightCycler® technologies)
- array technologies
- methylation detection
- Next Generation Sequencing

Sampling and storage of starting material

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

If using EDTA / citrate stabilized plasma, please centrifuge the tubes within three hours after sampling and freeze the plasma.

Prospective centrifuge conditions are at 1200 g for 10 minutes following a centrifugation at 4°C, 16000 g for 10 minutes (vide “Circulating Nucleic Acids in Early Diagnosis, Prognosis and Treatment Monitoring”, Peter P. Gahan, 2015, S.55). Other centrifugation conditions are possible, optimized on specific equipment.

Cell-Free DNA BCT® tubes (Streck) should be centrifuged accordingly the instructions of producer. Freeze the plasma/serum at -80°C for long-term storage.

The kit is appropriable for preparation of free circulating DNA from serum or plasma but not from whole blood.

Invitek Molecular will not take responsibility if other sample types than described above are used or if the sample preparation advices are modified.

Principle and procedure

The **InviMag® Free Circulating DNA Kit/ IG** procedure comprises following steps:

- protein digestion
- binding of the DNA to the magnetic beads
- washing the bead bound DNA and evaporation of ethanol
- elution of DNA

Lysis

Samples are lysed at elevated temperatures in the presence of **Lysis Buffer HLT** and **Proteinase S**.

Binding of the circulating DNA

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

Removing residual contaminants

Contaminants are efficiently removed while the DNA remains bound to the magnetic beads.

Elution

The DNA is finally eluted in **Elution Buffer M**. The eluted DNA is ready-to-use in different subsequent downstream applications.

Yield and quality of circulating DNA

The amount of purified DNA in the **InviMag® Free Circulating DNA Kit/ IG** procedure from serum or plasma depends on the content, the sample source, transport, storage, and age of samples.

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 Invitek Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the Invitek Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 6). Do not use damaged kit components, since their use may lead to poor kit performance.

- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles
- Discard contaminated gloves immediately
- Do not combine components of different kits
- Avoid microbial contaminations 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

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

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

8 x 12 extractions
Add 100 ml of 96-100 % Ethanol to each bottle **Wash Buffer R2** and mix thoroughly. Fill 50 ml of 96-100 % Ethanol into each empty bottle **Ethanol.** Fill 40 ml 99.7% Isopropanol (molecular biologic grade) into each empty bottle **Binding Buffer RV.** Resuspend each **Carrier RNA Tube** in 1800 µl RNase free Water, solve the Carrier by 30 s vortexing (check for precipitates) and transfer the fluid in a provided empty tube, which is signed with **“Carrier RNA Solution”.** Each tube with “Carrier RNA solution” is enough for two runs with each 12 samples. Please store the non-used tube at -20°C until the next run.

Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Pipette tips
- Disposable gloves
- Vortex
- 96-100 % ethanol
- Isopropanol*

*) The **InviMag® Free Circulating DNA 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 tubes for the plasma samples are tubes with a diameter between 11 and 13 mm with a maximum length of 100 mm, take care that the tube material does not bind DNA.

Important indications

Using this kit, you have several options, which lead into different extraction assays.

First is the option between automatically transfer or manual transfer of samples into the Incubation Plate D.

To prevent loss of the sensitive cell free DNA we recommend to put the samples directly in the Incubation Plate D without sample transfer via InviGenius® (assays marked with ”MT” means “Manual Transfer”). Please do this immediately before starting the Extraction Process.

If using stabilized serum or plasma, (for example Cell-Free DNA BCT® tubes from Streck)

It is also possible to do an automatically transfer via InviGenius®. The automatically transfer of 12 samples takes approximately 30 minutes.

Secondly, there is the option to select between different elution volumes.

Possible eluate volumes are 40 µl, 80 µl or 300 µl. If you prefer a high DNA concentration then use a lower elution volume (for example 40 µl). If prefer a high DNA yield then use a higher elution volume (for example 300 µl).

An additional option is the concentration of 300 µl eluate via columns from Sartorius (Vivacon® 500; 30 MWCO). By this way the DNA concentration increases up to 1, 5 fold in comparison to an elution in 40 µl via the respective assay DCF_E40S4000_MT or DCF_E40S4000_AT. The reason is to minimize loss of yield via the dead volume, which is constant in relation to the variable elution volume, means the higher the elution volume the lower is the loss via dead volume. After a high volume elution (300 µl) you can concentration elevate via spin column concentration.

For this additional special protocol, see Appendix 1.

Summary of possible assays using this kit

Assay	Sample transfer	Sample volume to be provided	Eluate Volume
DCF_E40S4000_AT	with	4500 µl	40 µl
DCF_E40S4000_MT	without	4000 µl	40 µl
DCF_E80S4000_AT	with	4500 µl	80 µl
DCF_E80S4000_MT	without	4000 µl	80 µl
DCF_E300S4000_AT	with	4500 µl	300 µl
DCF_E300S4000_MT	without	4000 µl	300 µl

1. Minimum volume of samples

The procedure of the **InviMag® Free Circulating DNA Kit/ IG** is optimized for the isolation of circulating DNA from up to 4 ml serum or plasma. If using sample transfer via InviGenius® it is advised to provide at least 4.5 ml in the sample tube to prevent pipetting distribution errors during processing (more if the tube diameter is bigger than 12 mm).

2. Sample volume smaller than 4 ml

Dilute samples with a volume smaller than 4 ml with physiological saline solution physiological saline solution (0.9 % w/v NaCl) or PBS to a volume of 4 ml, if starting in the Incubation Plate D without sample transfer.

Dilute samples with a volume smaller than 4.5 ml with physiological saline solution physiological saline solution (0.9 % w/v) or PBS to a volume of 4.5 ml, if starting with automated sample transfer (more if the tube diameter is bigger than 12 mm).

3. Elution volume

The final processed sample volume is 4 ml, which is eluted in different volumes of Elution Buffer M depended on specific assay:

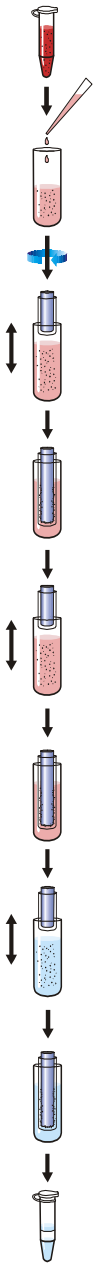
40 µl Eluate (Assays: DCF_E40S4000_AT or MT)
80 µl Eluate (Assays: DCF_E80S4000_AT or MT)

300 µl Eluate (Assays: DCF_E300S4000_AT or MT)
will be transferred to the Elution Plate E (compare table above).

Prevention of cross-contamination

To comply with the demanding guidelines of *in-vitro* diagnostics we programmed the InviGenius® PLUS to route the pipettor in such a way that possible contamination-risks are minimized.

Scheme of the InviMag® Free Circulating DNA Kit/ IG

Add the sample tubes in the sample loading rack or start directly from Incubation Plate D with prefilled samples. Add the Buffers in the Buffer loading rack.	
	4 ml sample is mixed with 4 ml **Lysis Buffer HLT,** 190 µl **Proteinase S** and 30 µl **Carrier RNA Solution**. Incubation at elevated temperature is performed for 10 min. 5.76 ml Binding Buffer RV and 200 µl MAP Solution B are added to the lysate. DNA binds to magnetic particles Magnetic separation Washing of the particle fixed DNA Magnetic separation Elution of DNA in Elution Buffer M in the selected volume Magnetic separation and removal of MAP Solution B Transfer of pure DNA in Elution Plate E

Preparing the samples for processing on the InviGenius® PLUS

This step should be carried out after the complete preparation of buffers and loading of InviGenius® to avoid degradation of DNA in samples. It should be the last step before starting the assay on InviGenius®.

Avoid thawing samples more than once.

The Kit is usable for several assay scripts (compare “Important indications”).

Assays marked “AT” include the sample transfer from sample tubes to Incubation Plate D.

Assays marked “MT” start direct from Incubation Plate D in which the samples put by the user.

Select the assay suitable for your purpose.

Add the samples into the Incubation Plate D (exactly 4 ml each optionally fill to 4 ml).

After adding of samples the Incubation Plate D is placed into the Incubator. Name the samples by first loading an empty sample rack and editing the sample names manually.

Only samples with names will be processed!!!

General overview of the InviGenius® PLUS

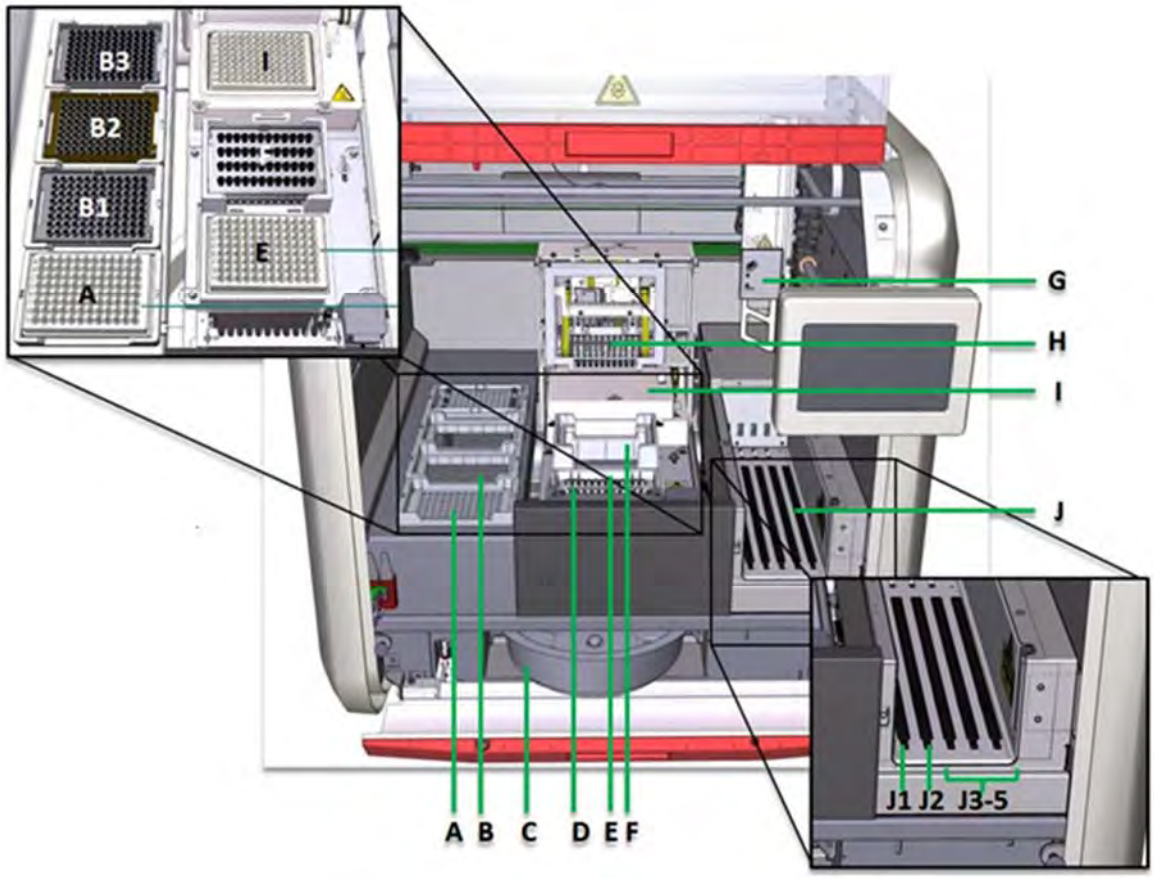


Figure 1: Frontal view of the InviGenius® PLUS system

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 (must be ordered separately from Invitek Molecular GmbH) **C** is located on the lower side of the **InviGenius® system** behind the red flap. The waste shaft **D** is completely stainless steel and easily removable for autoclaving.

The loading bay is divided into sample loading bay **J1,** eluate loading bay **J2** and reagent loading bay **J3-5**. 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® PLUS

Preparing the reagents

Add 100 ml of 96-100 % Ethanol to each bottle Wash Buffer R2 and mix thoroughly.

Fill 50 ml of 96-100 % Ethanol into each empty bottle Ethanol.

Add 40 ml 99.7 % Isopropanol (molecular biologic grade) to each bottle Binding Buffer RV directly before the run.

Resuspend each **Carrier RNA Tube** in 1800 µl RNase free Water, solve the Carrier by 30 s vortexing (check for precipitates) and transfer the fluid in a provided empty tube, which is signed with **"Carrier RNA Solution".**

Each tube with "Carrier RNA solution" is enough for two runs with each 12 samples. Please store the non-used tube at -20 °C until the next run.

Please take care to use the accurate filling volumes even for the empty bottles because the instrument is checking filling levels of the buffer bottles and vials.

Preparing the system

Turn on the InviGenius® PLUS using the power switch located on the right back side of the instrument. Keep the door of the InviGenius® PLUS closed during initialization.

After Initialization of the InviGenius® PLUS a login screen appears (Figure 2).

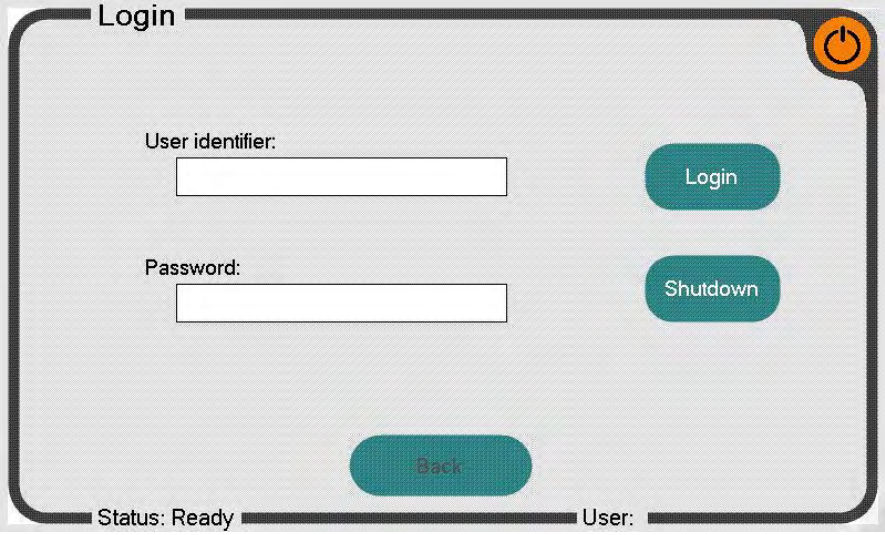


Figure 2: Login screen of the InviGenius® software

Log in with the provided user name and password.

After Login the main menu of the InviGenius® software is shown (Figure 3).

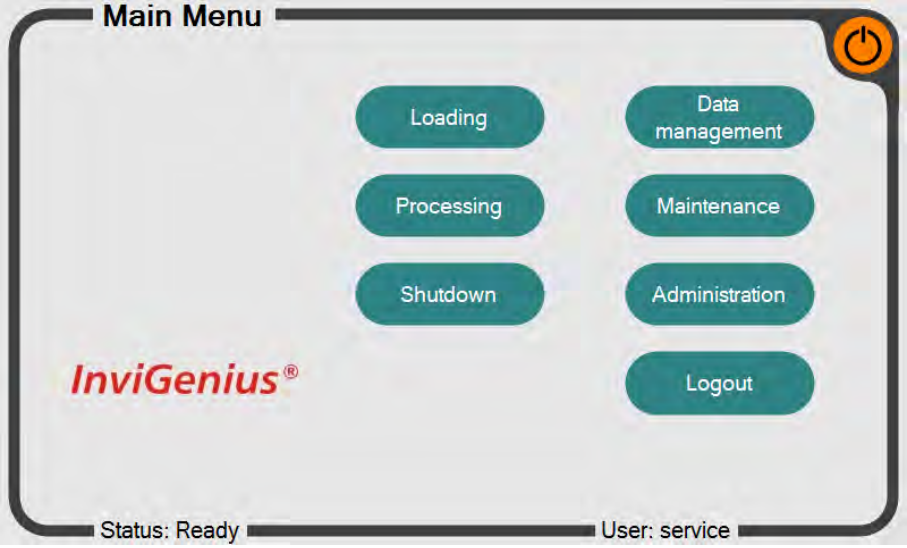


Figure 3: Main menu of the InviGenius® software

Select “Loading” to start with loading of the system.

Select “Processing” to define and run an assay if the system has already been loaded.

Sample loading:

After selecting “Loading” the loading screen appears (Figure 4).

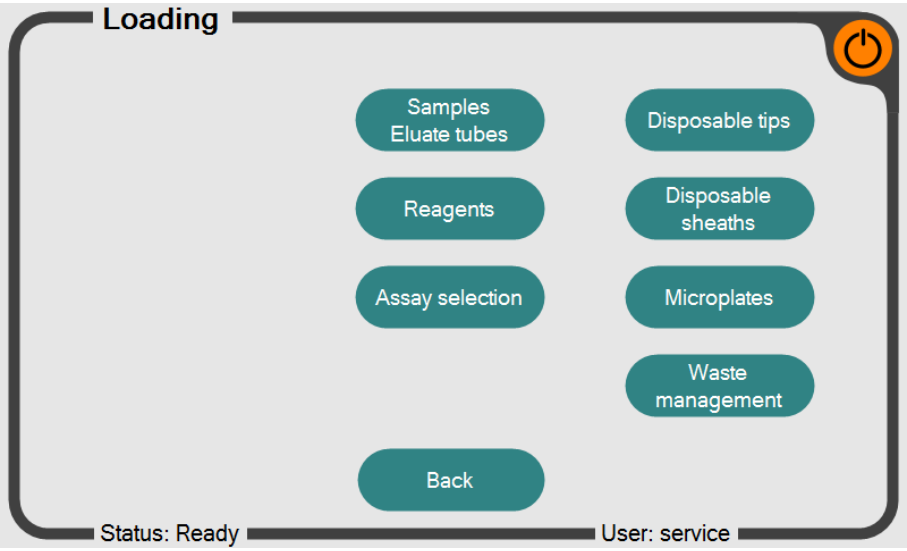


Figure 4: Loading screen of the InviGenius® software

Select “Samples Eluate tubes” to proceed with the sample loading.

The Sample loading screen appears (Figure 5).

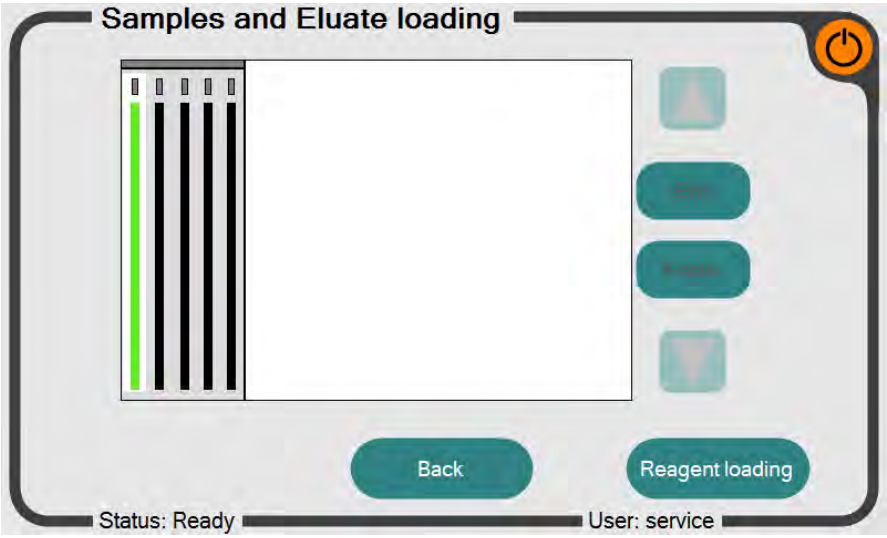


Figure 5: "Sample and Eluate loading" screen of the InviGenius® software

Please add the samples to the rack. Please decap the tubes before transfer to the loading rack.

Primary tubes should be used directly as sample tubes. If the samples are not provided in primary tubes, please transfer them into appropriate tubes. Sample tubes are not provided with the kit and can be ordered (5 ml tubes, 75x12 mm, PP) or see recommendation at page 9, chapter: reagents and equipment to be supplied by user.

For each reaction, a sample volume of 4 ml is processed. However, it is recommended that the total sample volume filled in the sample tubes should be at least 4.5 ml (in 12 mm diameter tubes; bigger tubes need a greater dead volume; 4.5 ml in 13 mm tubes up to 5.5 ml in 16 mm tubes) to ensure stable processing. Please take care, that only the first 12 positions of the sample rack can be processed. For correct identification of the sample tubes, the unique bar codes must face the barcode scanner located at the right side of the loading bay.

If using assays without sample transfer (marked with MT) do the same without samples, and name the samples loaded in the Incubation Plate D from left to right via the following process.

The following screen appears if the barcode is not readable or no barcode is provided (Figure 6).

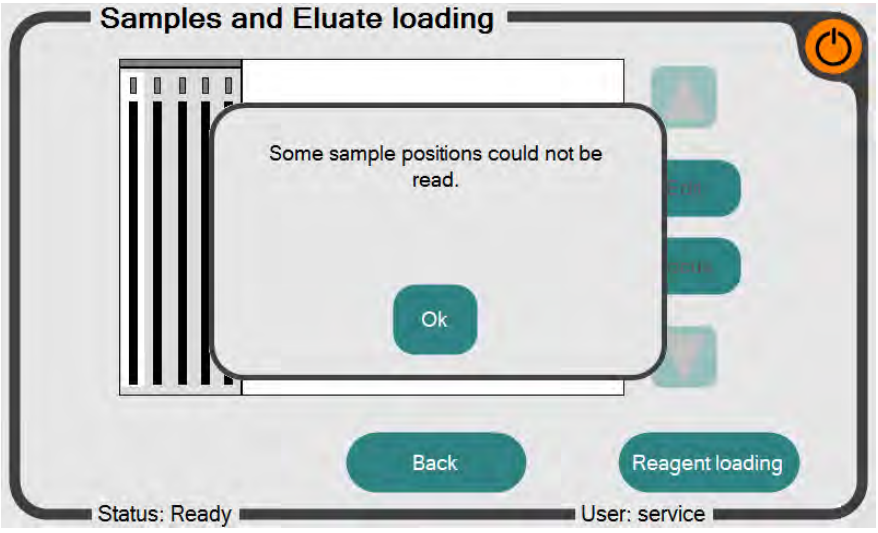


Figure 6: "Sample and Eluate loading" screen of the InviGenius® software

Press "Ok" and "Edit". Scan each sample using the provided hand barcode scanner or edit them manually.

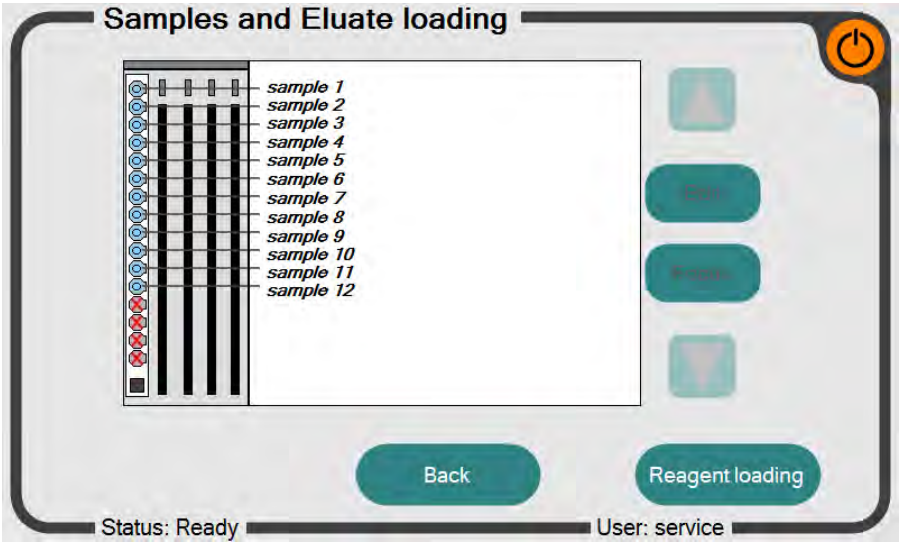


Figure 7: "Sample and Eluate loading" screen of the InviGenius® software with 12 named samples

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 7). In case of unsuccessful sample identification, remove the rack, check the bar code orientation and reinsert the rack slowly. It is also possible to rename the samples by selecting the corresponding sample by using the arrow fields and pressing the "Edit" button.

After a certain time (about 5 min) the bar code scanner will be inactivated. In that case, the user has to restart the scanner with the "Focus" button if the loading procedure is not finished.

The lane right of sample rack will be highlighted after a successful loading of the samples. This indicates that the InviGenius® is now ready for the insertion of the eluate rack at this position.

If you want to use the provided eluate plate (Eluate Plate E) which is provided in the kit contents proceed with "Reagent loading" on the bottom right of the screen.

Alternatively you can use barcoded Eluate tubes (not provided; e.g. 2 ml Cryotubes from Sarstedt). Please make sure you use screwcaps as Eppendorf caps may interfere with the pipettor movement. Eluate tubes are useful for planned long-term storage of the eluates (biobanking, etc.).

Eluate loading:

Insert the eluate rack.

(If a screen with "Wrong rack type character detected" appears repeat the insertion of the eluate rack. If the message persists, you are probably using the false rack. Please make sure you are using the correct rack).

After a successful eluate rack loading you are reminded to unload the eluate plate (Figure 8).

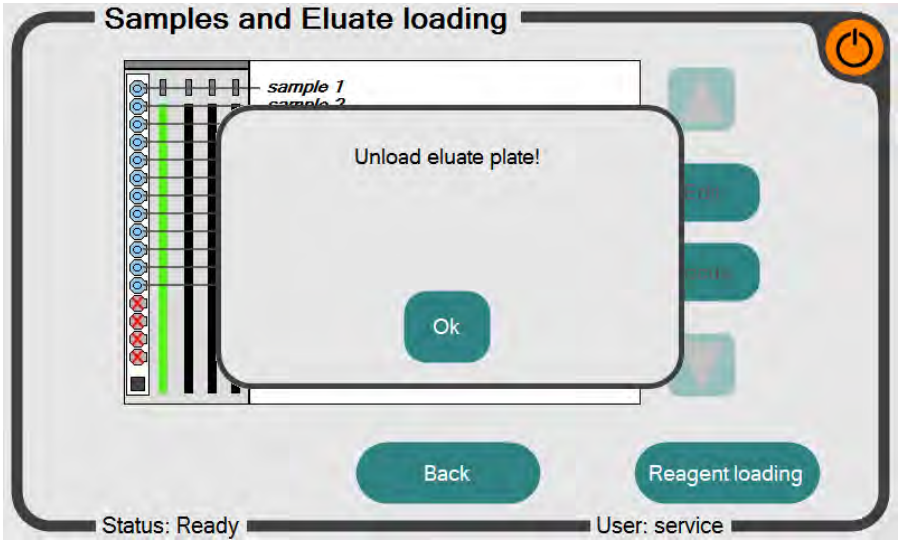


Figure 8: Screen of the InviGenius® software to unload eluate plate

Remove the Eluate plate if loaded and press “Ok”.

The following screen appears (Figure 9) with the barcodes of the eluates.

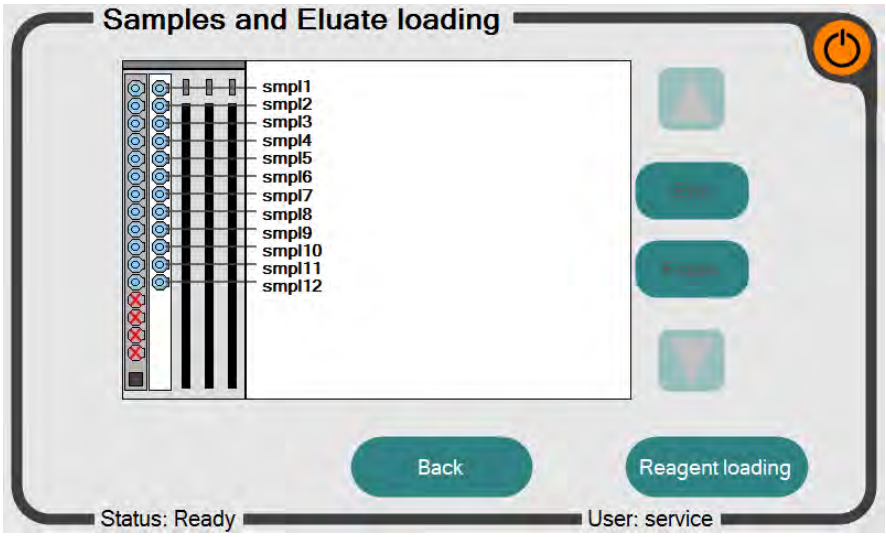


Figure 9: “Sample and Eluate loading” screen of the InviGenius® software

After successful loading of the samples and/ or elution tubes 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 10).

Insert all provided reagents into the provided reagent rack of the InviGenius® system. Take care 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 relevant as the type and position of a reagent container is identified by the unique bar code. However, the possible loading positions are limited by the size of corresponding bottles.

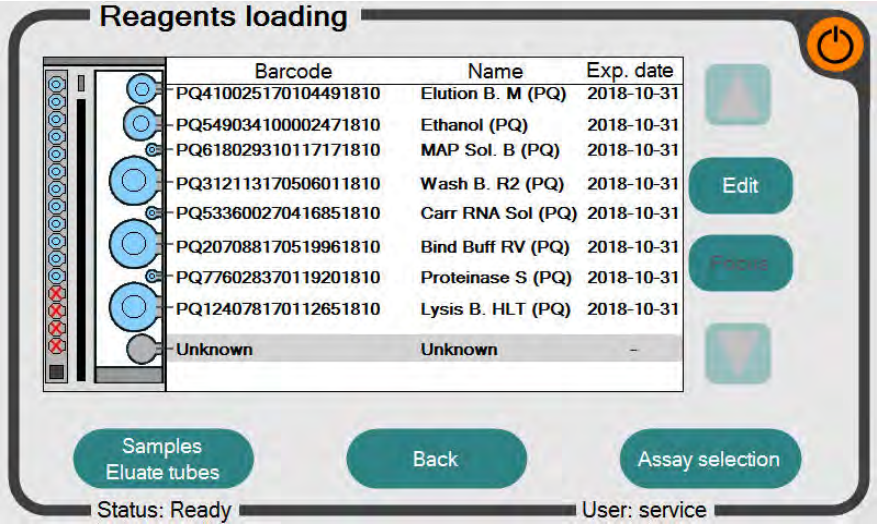


Figure 10: "Reagent loading" screen of the InviGenius® software

After rack insertion, the loading status of the reagents will be shown. In case of an unsuccessful reagent allocation, remove the rack completely, check bar code orientation and repeat the insertion procedure slowly.

Assay Selection:

Press the bottom “Assay selection” and select the assay.

Proceed with disposable tip loading.

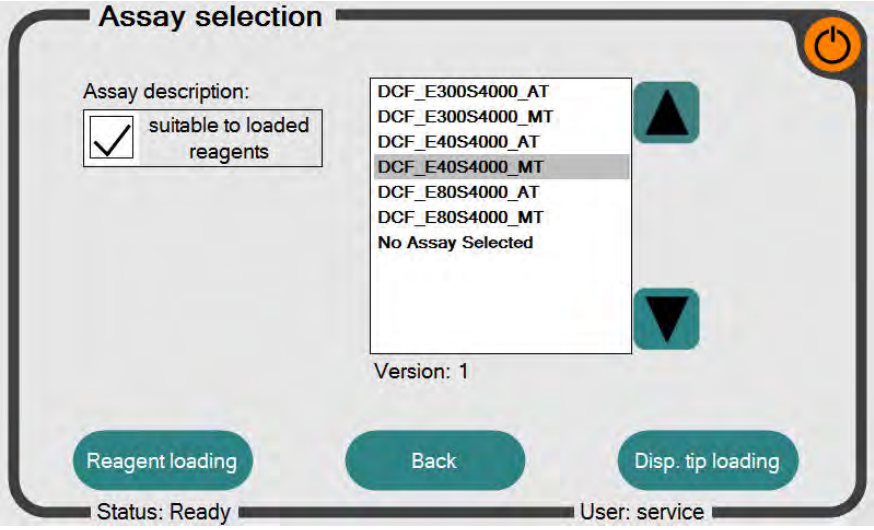


Figure 11: “Assay-selection” screen of the InviGenius® software

If no Assay is offered, please go back to the Reagent loading screen by pressing the corresponding button on the bottom left of the screen. Check again all needed reagents are loaded and correctly identified by the barcode. If the problem persists please make sure the assay is registered in the Administration Screen of the InviGenius® PLUS.

Disposable tips Loading:

Press the bottom “Disp. tip loading”.”

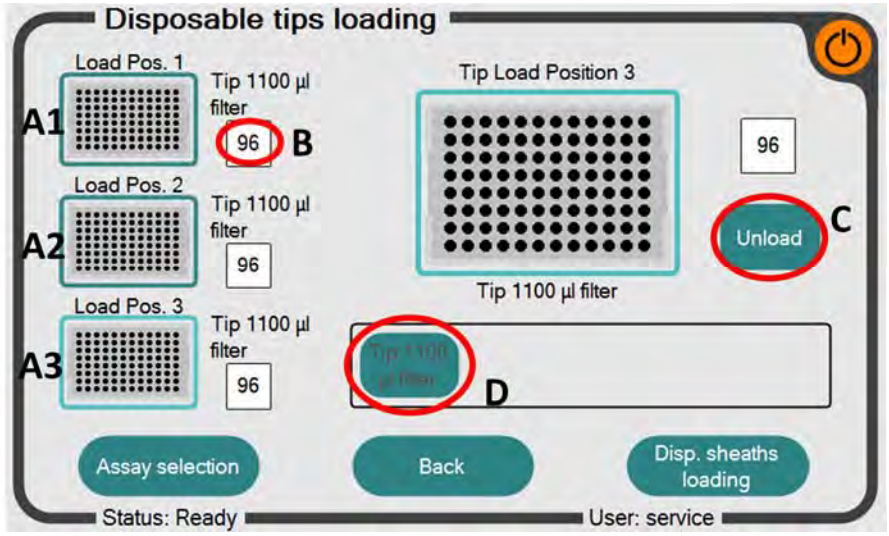


Figure 12: Disposable tip loading screen of the InviGenius® software

There are three tip rack positions on the InviGenius® system (Fig. 12, A1-A3). Remaining tip-numbers are shown in 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 (A1 or A2 or A3), the software will focus this 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 on D

For the **InviMag® Free Circulating DNA Kit/ IG** only 1100 µl filtered tips must be used.

Attention: *It is very important to allocate the type of tips correctly in the software that have been loaded into the instrument. In case of false tip allocation, overfilling of the tip may destroy the pipettor head!*

All protocols should be used in combination with filter tips to ensure efficient prevention of sample or reagent cross-contaminations. Invitek 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 filter tips, which can be ordered at Invitek Molecular. Invitek Molecular offers 1100 µl Conductive filter tips (10x 96 pieces, order no. 5011120200). Be sure that Conductive filter tips are used otherwise the tip detection unit, installed in the pipetting unit, will reject the tips and no run will be possible.*

Disposable Sheaths Loading:

Press the bottom “Disp. Sheath loading”. The sheaths are used as protection devices for the magnetic rods.

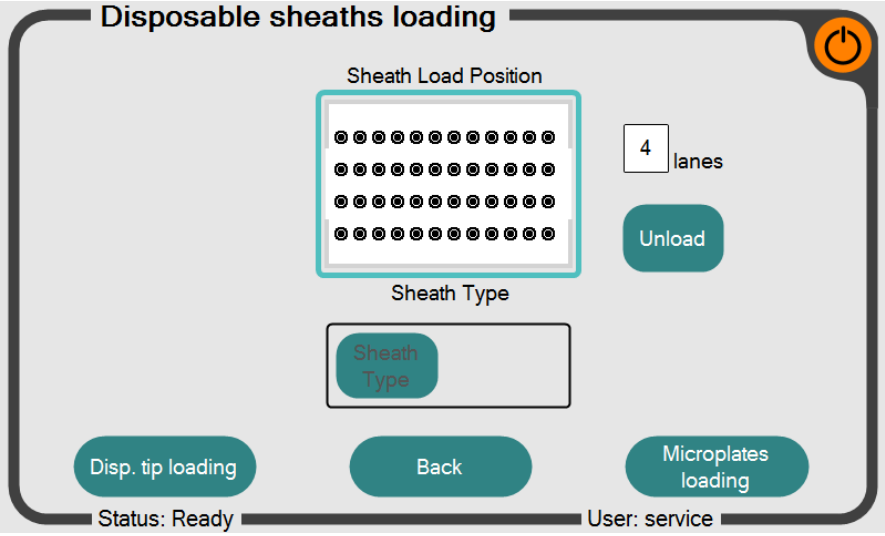


Figure 13: Disposable sheaths loading screen of the InviGenius® software

The loading procedure of the disposable sheaths works analogous to the disposable tip loading screen. One run consumes 12 disposable sheaths (one row in the sheaths rack) are used, regardless of processed sample numbers, assuring that the rods are always protected against contaminations.

In general, the number of sheaths supplied within the kit is sufficient for the number of runs printed on the kit package. If you are lacking sheaths due to lower sample numbers than 12, sheaths can be ordered separately at Invitek Molecular (100 pieces bulk, order no. 5011120300 or 10 x 48 pieces, order no. 5011120400).

Comparable to 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. A sheaths detection sensor is installed in the device. If less than 12 sheaths picked up by the instrument a warning will be displayed and all picked up sheaths will be discarded into the waste before a next row of sheaths will be picked up for testing.

To avoid contaminations, we strongly recommend not washing/reusing any disposed sheaths!

Plate Loading:

Press the bottom “Microplates loading “. Analogous to the previous loading screens, the Incubation Plate D, Working Plate B and Elution Plate E (if no eluate rack) are loaded within the plate loading screen (Figure 14).

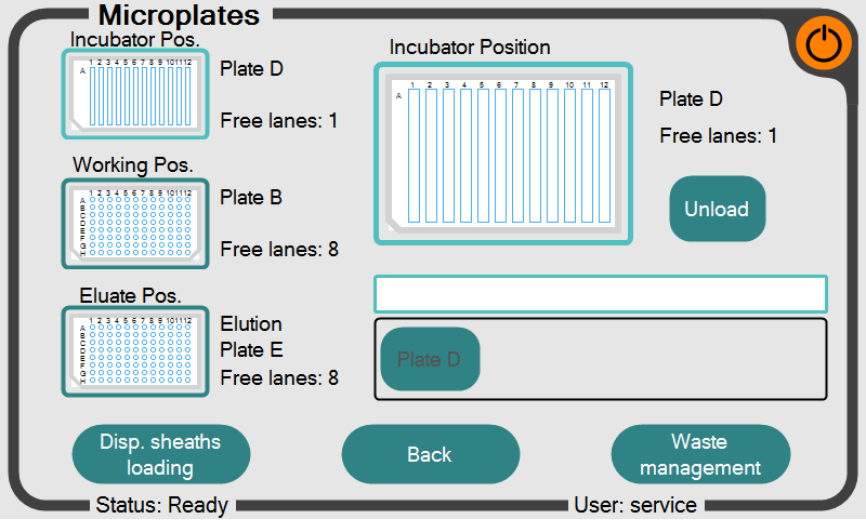


Figure 14: Plate loading screen of the InviGenius® software

In general, the Incubation Plate D and Working Plate B are used at the incubator and working position whereas at the eluate position the Elution Plate E is used.

Used plates can be unloaded and reloaded by:

1.) Press the plate position directly. The software will focus at the plate position on the main screen (in Figure 12 Incubation plate D is focused).

2.) Press the “Unload” button right from the main screen

3.) Load a new plate by pressing on the offered plate placed beneath from main screen

For a successful run, the InviGenius® needs new plates in the incubator position and in the working position and one free lane in the eluate position or an eluate rack.

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

To avoid contaminations, we strongly recommend not washing/reusing disposed plates!

Proceed with the bottom “Waste management”.

Waste management:

Please make sure that the waste tray capacity is sufficient for your planned assay. If not, empty the solid waste. Insufficient waste capacity is displayed in the batch checking screen.

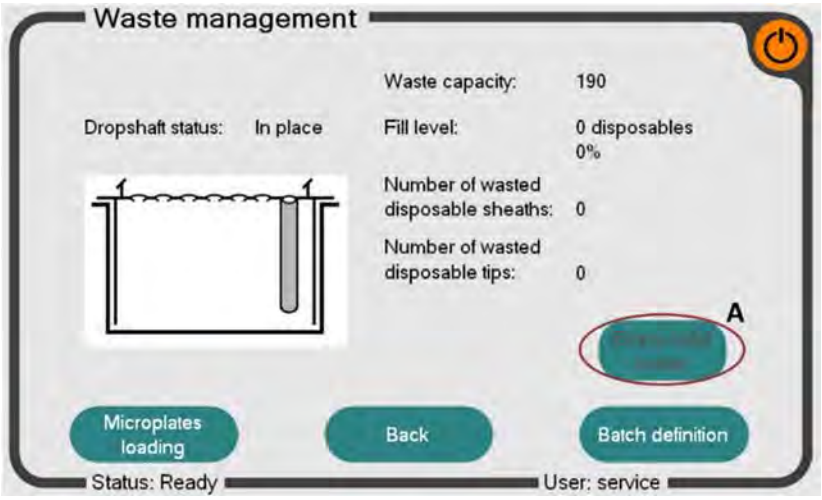


Figure 15: Waste-management-screen of the InviGenius® software

If you have cleaned, the waste tray (must be ordered separately from Invitek Molecular GmbH), please use the “Empty solid waste” button (A).

Batch definition:

Press the bottom “Batch definition”. Please select the appropriate assay and check the samples you want to process in this run.

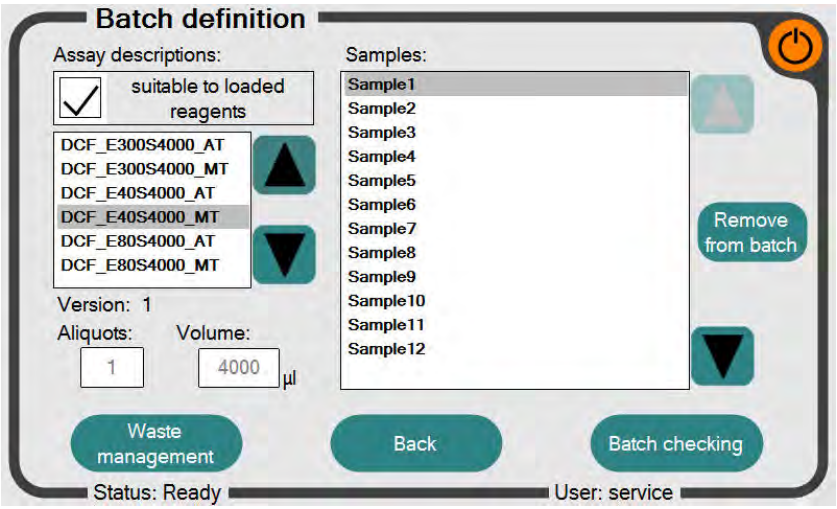


Figure 16: Batch-definition-screen of the InviGenius® software

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 (left of screen).

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 and clicking on the “Remove from batch” button (right of screen).

If you loaded the samples by manual transfer **do not exclude samples! This would lead to logical confusions in the run.**

Batch checking:

Press the bottom “Batch checking”. This screen shows a summary of all disposables, samples and reagents in one informational screen.

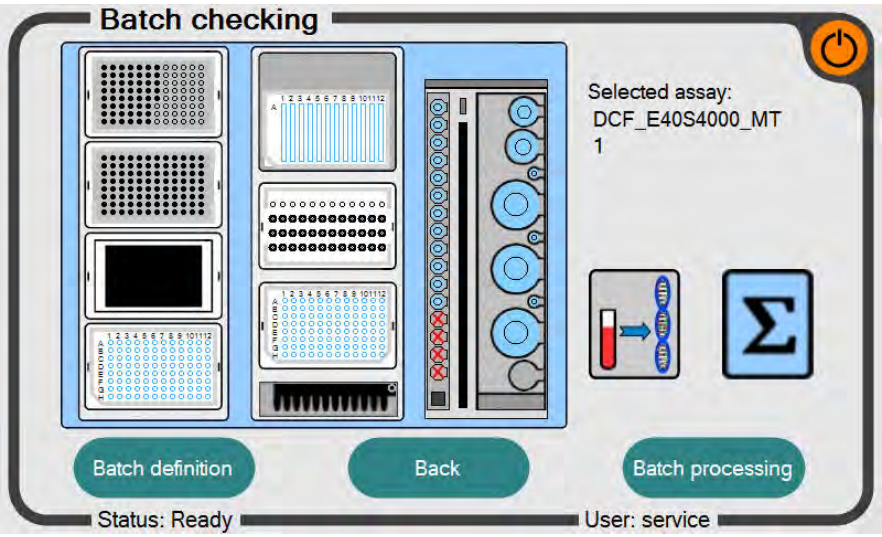


Figure 17: Batch-definition-screen of the InviGenius® software

Please make sure that all required components are loaded correctly. In case of any error, the position with the problem will be highlighted red. To solve any error, click on the red highlighted field and follow the instructions printed on the instrument screen.

If no errors during the loading steps occurred, proceed by pressing the button “Batch processing”.

If using assay without automatic sample transfer (marked MT) now put the Incubating plate D containing samples in the InviGenius®!

Batch processing:

Press the bottom “Batch processing”.

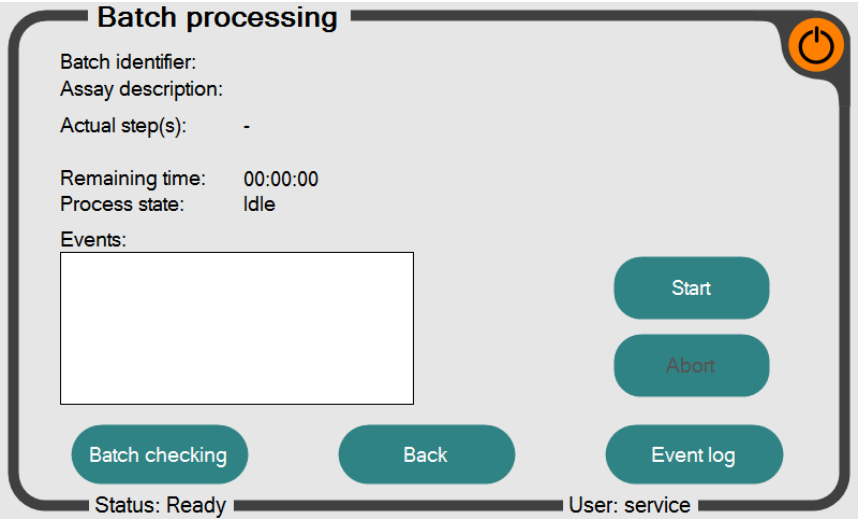


Figure 18: Batch-processing-screen of the InviGenius® software

After closing the system-door, the assay can be started by pressing the “Start process”-Button. The “check the elution hedgehog” appears (Figure 19).

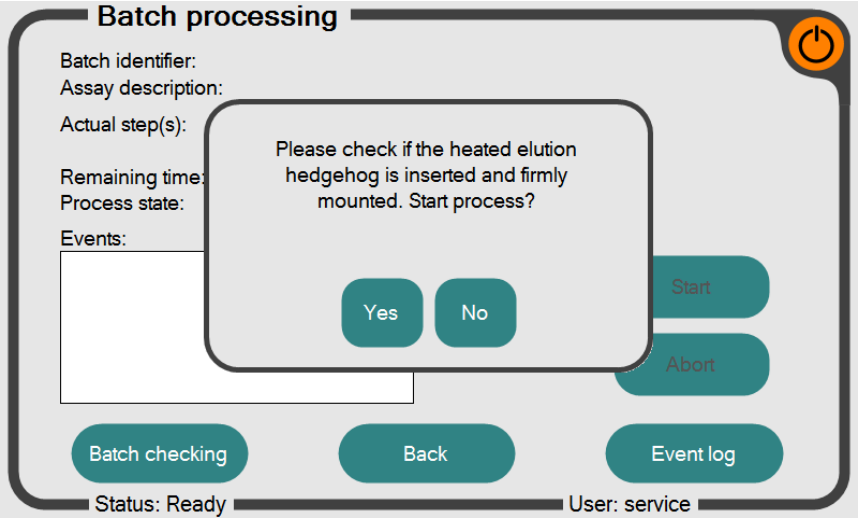


Figure 19: last check-screen of the InviGenius® software

Press “yes” to continue or “no” to stop and insert the hedgehog.

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 open the door by force during a run. This will cause an interruption of the run!

At the end of the run, the DNA-containing eluate is located in the appropriate eluate position and can be used for further applications. The eluate should be frozen to further applications (-20°C or -80°C).

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 infectious.

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 select “Maintenance” on the main menu of the InviGenius® software.

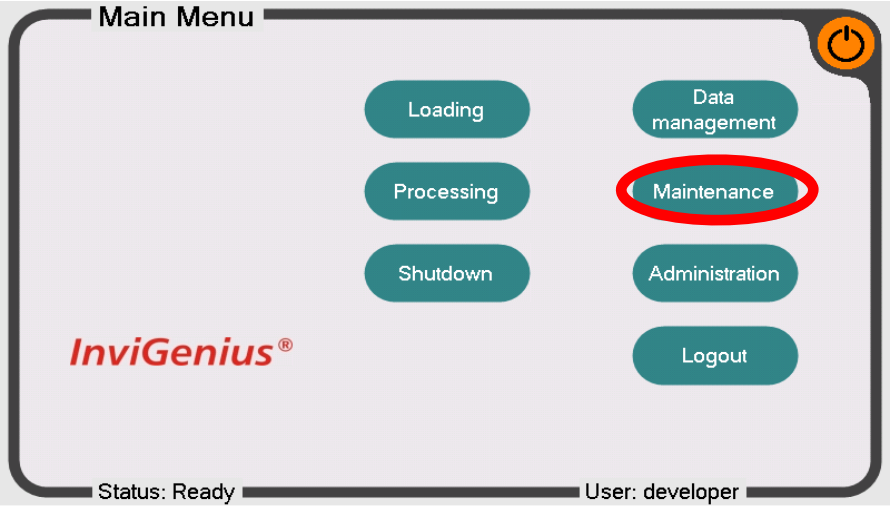


Figure 17: Main menu of the InviGenius® software

Select “UV decontamination” in the sub folder “Maintenance”.

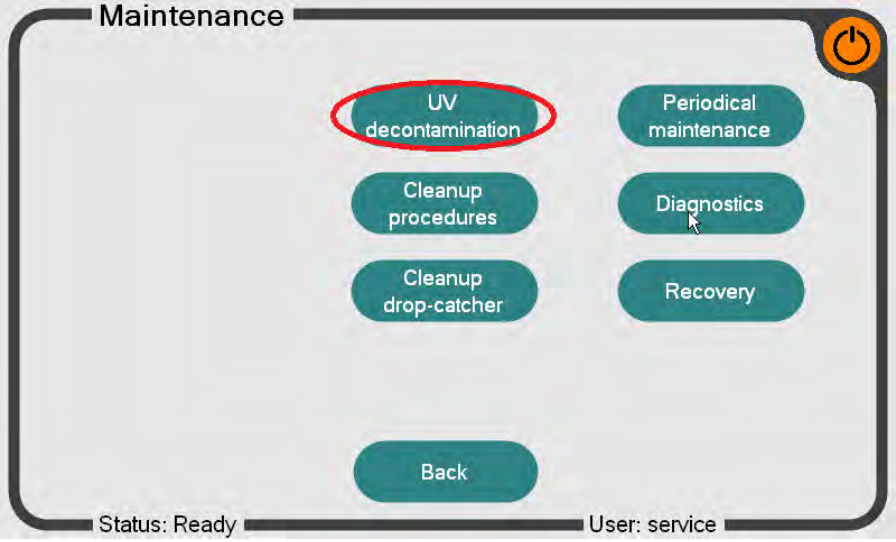


Figure 18: Maintenance screen of the InviGenius® software

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

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 personnel is exposed to direct UV light. Do not try to open the instrument door by force during the decontamination process.

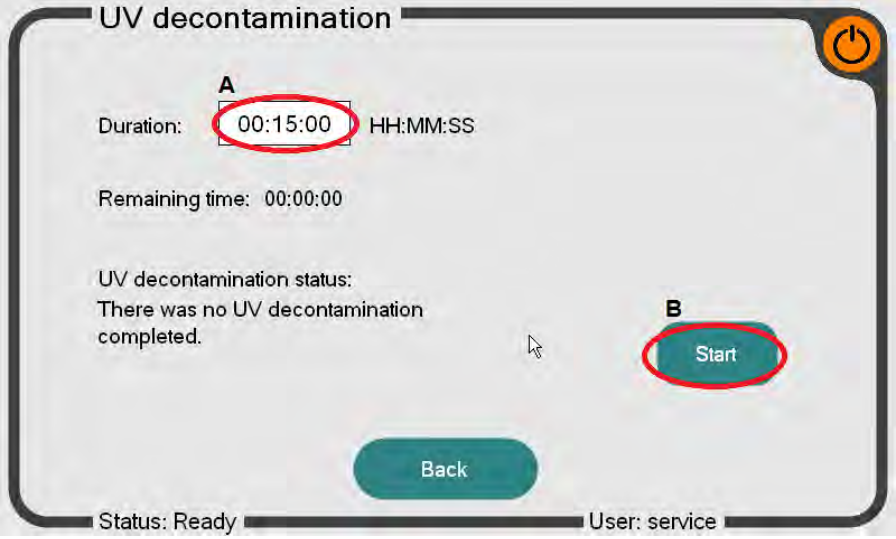


Figure 19: UV decontamination screen

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

Appendix 1

Protocol: Concentrating DNA with Vivacon® 500 columns

Required material

- Eluates of Invigenius assays with 300 µl elution volume (DCF_E300S4000_MT or AT)
- VIVACON 500 concentrators and collection tubes (Sartorius; VIVACON® 500, Membrane: 30,000 MWCO HY; REF: VN01H22)

Concentration

Compare also “Technical data and operating instructions” of VIVACON® 500 by Sartorius.

- Insert a concentrator in collection tube
- Fill concentrator with eluate (300 µl optimal)
- Close collection tube and insert assembled concentrator into centrifuge.
- Centrifuge at 5.000 g for 20 minutes.
- Empty filtrate container and transfer the concentrator in a new collection tube (concentrator not invert!)
- Fill 40 µl DNase / RNase free Water (pre-warm 60°C) in concentrator for recover DNA
- Close collection tube and insert assembled concentrator in thermomixer for 5 minutes at 60°C and 500 rpm
- Open the collection tube, invert the concentrator body into the same tube and insert assembled concentrator into a centrifuge.
- Centrifuge at 2500 g for 2 minutes.
- Empty concentrator and close collection tube containing concentrated DNA

Note: For higher concentration of DNA, you may use a lower volume of DNase / RNase free water, down to 20 µl. Keep in mind that yield may be reduced for a small part, if the elution volume is reduced. Reducing further, the elution volume will lead to dramatic losses of yield.

Appendix 2

Storage of DNA

Circulating DNA should be stored at – 20 C. Avoid repeated freezing and thawing cycles.

Example data

Samples shown are clinical samples from patients suffering from different kinds of gynecological cancers. Samples were prepared from rinse liquids (1-3), plasma (4, 5) and serum samples (6-15). Preparation of these samples were done with InviMag® Free Circulating DNA Kit/ IG using the InviGenius® PLUS robotic platform (IG Plus) and three different manual kits of competitor companies (A, B, C).

Sample volume was in the range from 200 µl up to 4 ml depending on the kit instructions. Elution volume was 115 µl in all cases.

Quantitation of the circulating DNA was performed with a real-time PCR of actin. The numbers of actin copies were normalized to the equivalent volume of 1 ml sample.

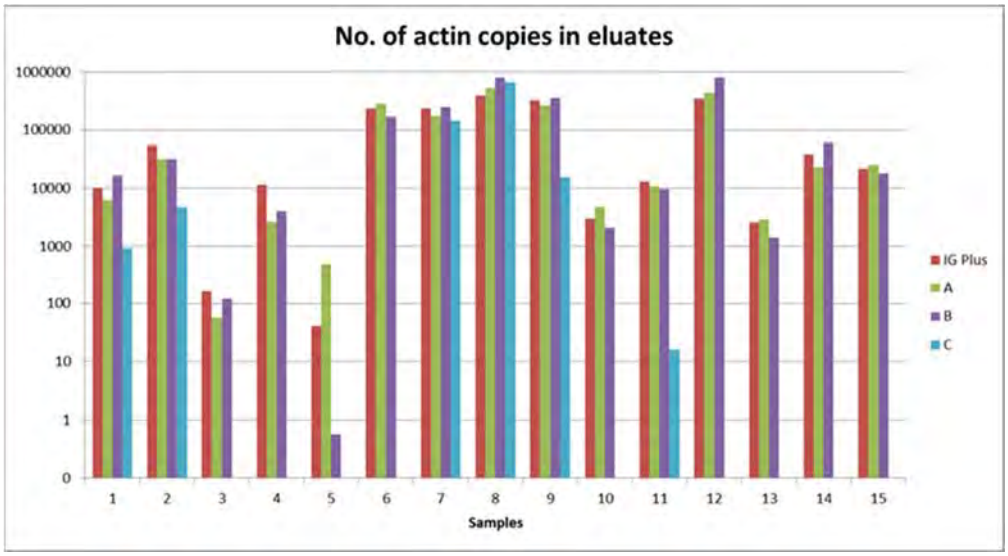


Fig. 1: Copies of actin were reassessed after bisulfite conversion (BS) to evaluate the quality and purity of the eluates.

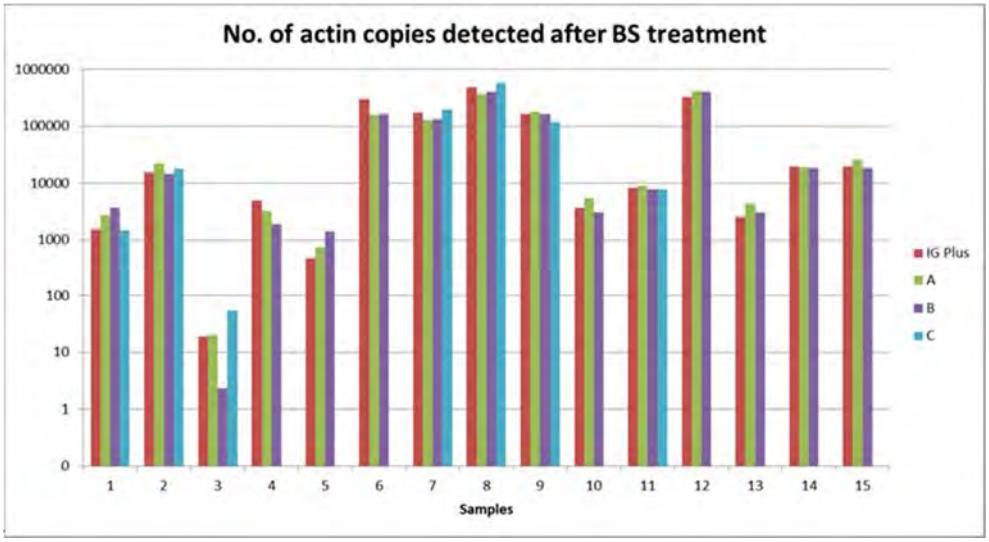


Fig. 2: The data shows the comparability of the different methods. The InviMag® Free Circulating DNA Kit/ IG using the InviGenius® PLUS robotic platform (IG Plus) is more reproducible and safe than the other kits. The process is completely user independent, and with nearly no hands on time.

Troubleshooting

Problem	Probable cause	Comments and suggestions
Pipetting distribution errors	Samples transfer failed	The sample tube must contain at least 4.5 ml sample (up to 5.5 ml in tubes with bigger diameter (≥13 mm)
		Pipet tip partially clotted, automatic removal of samples with clots.
	Reagent / buffer transfer failed / incomplete	Ensure that the **Wash Buffer R2** and **Ethanol** are filled up properly with ethanol and **Binding Buffer RV** is filled up properly with isopropanol Ensure that **Carrier RNA Solution** is filled with 850µl RNase Do not reuse bottles more often than described
Low concentration of extracted DNA	Incorrect storage of starting material	Ensure that the storage condition of the starting material was correct Avoid multiple freezing and thawing cycles of the sample
	No / too much ethanol / isopropanol added to **Wash Buffer R2 / Binding Buffer RV** or **Ethanol**	Ensure that the **Wash Buffer R2** and **Ethanol** are filled up properly with ethanol and **Binding Buffer RV** is filled up properly with isopropanol
	No **Carrier RNA Solution** in the tube filled	Ensure that **Carrier RNA Solution** is filled with 850µl from **Carrier RNA**)
Degraded DNA	Incorrect storage of starting material	Ensure that the storage conditions of the starting material was correct Avoid multiple freezing and thawing cycles of the sample
	Nature of the sample material	Content of free circulating DNA in Serum or Plasma can be very small, DNA always is fragmented
No assay selectable	Combination of reagents from different kits or blocked barcode during reagent loading procedure, or reagents are missing.	Assure that only reagents belonging to one kit type are used. A combination of reagents belonging to different kit types is not supported by the system. Ensure that the reagent barcode label is visible within the reagent rack window
Eluted DNA is brownish colored	Small part of the magnetic particles are left in the elution	Centrifuge the eluates at full speed for 1 min and transfer supernatant to a new plate / tube. In normal processing this should not occur, check teaching of the instrument via service.

Ordering information

Product	Package size	Catalogue No.
InviMag® Free Circulating DNA Kit/ IG	8 x 12 preps	2439320400

InviGenius®PLUS and consumables

InviGenius®PLUS	1 unit	5011100000
Starting Box I/ IG: Sheath Box Conductive filter tips, 1 ml; 2 x 2 rack/ pack (384 pieces) 5 Waste Trays 120 sample tubes	1 box	2400110100
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

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