

USER MANUAL

InviMag[®] Bisulfite Conversion Kit/ IG

for fully automated bisulfite conversion of DNA (human genomic DNA from different sources) with magnetic beads

Instruction for InviMag[®] Bisulfite Conversion Kit / IG

The **InviMag[®] Bisulfite Conversion Kit/ IG** is designed for fully automated bisulfite conversion and purification of a maximum of 5 µg DNA in a volume of up to 50 µl. 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 barcodes for samples and reagents avoid unwanted transpositions.

Due to the high purity, the converted DNA is ready for use in methylation analysis or can be stored at -20°C/ -80°C for subsequent use.

For research use only!

Trademarks: InviMag[®], InviGenius[®]. 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[®] 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[®], InviGenius[®] are registered trademarks of Invitex Molecular GmbH.

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

© 2020 Invitex Molecular, all rights reserved.

Contents

| | |
|---|-----------|
| Kit contents of InviMag® Bisulfite Conversion Kit/ IG | 3 |
| Symbols | 4 |
| Storage | 4 |
| Quality control | 4 |
| Intended use | 5 |
| Product use limitation | 6 |
| Safety information | 7 |
| Product characteristics of the InviMag® Bisulfite Conversion Kit/ IG | 8 |
| Principle and procedure | 8 |
| Important points before starting a protocol | 8 |
| Equipment and reagents to be supplied by user | 9 |
| Storage of starting materials | 9 |
| Yield and quality of bisulfite converted DNA | 9 |
| Preparing reagents and buffers | 9 |
| Prevention of cross-contamination | 10 |
| Scheme of the InviMag® Bisulfite Conversion Kit/ IG | 10 |
| Preparing the samples for processing on the InviGenius®-system | 11 |
| General overview of the InviGenius® System | 11 |
| Preparing and loading of the InviGenius®-system | 11 |
| After the run | 22 |
| Maintenance (UV sterilization) | 22 |
| Troubleshooting | 24 |
| Ordering information | 24 |

Kit contents of InviMag[®] Bisulfite Conversion Kit/ IG

| Component | 8 x 12 reactions | |
|---|--|---------------------------------|
| Catalog No | 3030200100 | |
| Lysis Buffer HL | 110 ml | 96 samples (in max. 16 runs) |
| SNAP Solution / IG | 8 x 1.5 ml | 12 samples (in max. 2 runs) |
| MAP Solution B/ IG | 8 x 1.2 ml | 12 samples (in max. 2 runs) |
| Solution D | 25 ml | 96 samples (in max. 16 runs) |
| Ethanol (fill with 96-100% Ethanol) | empty bottle (final volume 30 ml) | 96 samples (in max. 16 runs) |
| Wash Buffer II | 4 x 36 ml (final volume 4 x 120 ml) | 24 samples (in max. 4 runs) |
| Solution BS | 8 x 2 ml | 12 samples (in max. 1 run) |
| Solution BS/ IG | 8 (empty tubes) | 12 samples (in max. 1 run) |
| Elution Buffer | 30 ml | 96 samples (in max. 16 runs) |
| Incubation Plate A | 2 | 8 runs 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 | |
| Sealing Foils | 4 | |
| Incubator Stripe Foils | 2 | |
| | <p>Fill the Ethanol bottle with 30 ml Ethanol</p> <p>Add 84 ml Ethanol to each bottle Wash Buffer II.</p> <p>Before each run: Transfer the Solution BS with a pipet carefully to an empty Solution BS IG tube. Do not agitate the solution during transfer to minimize oxygen input.</p> | |

Symbols



Manufacturer



Lot number

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



Catalogue number



Expiry date



Consult operating instructions



Temperature limitation



Do not reuse



Humidity limitation

Storage

All buffers and kit contents of the **InviMag® Bisulfite Conversion Kit/ IG** should be stored at room temperature and are stable for at least 6 months.

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

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

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

Quality control and product warranty

Invitek Molecular warrants the correct function of the **InviMag® Bisulfite Conversion 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® Bisulfite Conversion 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® Bisulfite Conversion 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:

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

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

or contact your local distributor.

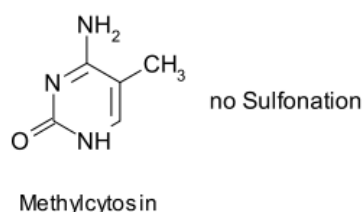
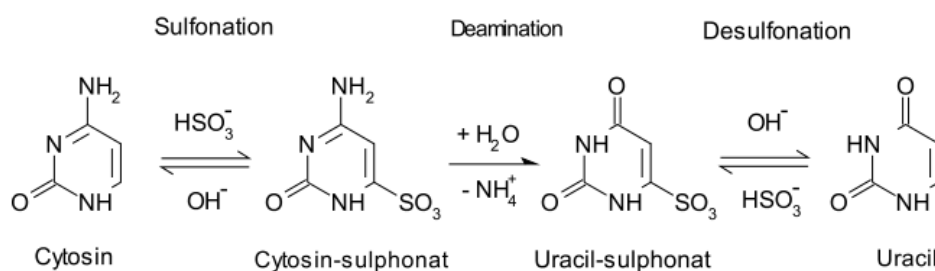
Intended use

The **InviMag® Bisulfite Conversion Kit/ IG** is designed for an automated DNA denaturation and bisulfite conversion processes of up to 12 samples.

DNA methylation is an important regulator of gene transcription and a large body of evidence has demonstrated that genes with high levels of 5-methylcytosine in their promoter region are transcriptionally silent, and that DNA methylation gradually accumulates upon long-term gene silencing. DNA methylation is essential during embryonic development, and in somatic cells, patterns of DNA methylation are generally transmitted to daughter cells with a high fidelity. Aberrant DNA methylation patterns – hypermethylation and hypomethylation compared to normal tissue – have been associated with a large number of human malignancies. Hypermethylation typically occurs at CpG islands in the promoter region and is associated with gene inactivation. A lower level of leukocyte DNA methylation is associated with many types of cancer. Global hypomethylation has also been implicated in the development and progression of cancer through different mechanisms. Typically, there is hypermethylation of tumor suppressor genes and hypomethylation of oncogenes.

In prokaryotes, DNA methylation provides a way to protect host DNA from digestion by restriction endonucleases that are designed to eliminate foreign DNA, or from destruction during repair events. In higher eukaryotes, DNA-methylation functions in the regulation/control of gene expression. It has been demonstrated that aberrant DNA methylation is a widespread event in cancer. A changed methylation pattern may be among the earliest changes to occur during oncogenesis. DNA methylation has also been shown to play a central role in gene imprinting, embryonic development, X-chromosome gene silencing, and cell cycle regulation. In eukaryotic CpG - DNA methylation consists of the addition of a methyl group to the fifth carbon position of the cytosine pyrimidine ring via a methyltransferase enzyme. The majority of DNA methylation in mammals occurs in CpG dinucleotides, but other methylation patterns do exist. In fact, 70 - 80 percent of all CpG dinucleotides in mammalian genomes are found to be methylated, whereas the majority of the twenty to thirty percent that remain unmethylated are within promoters or in the first exons of genes.

Nowadays efficiently and accurate DNA methylation analysis is essential for the study of cancer, gene expression, genetic diseases, as well as many other important aspects of biology. To date, a number of methods have been developed to detect/quantify DNA methylation including: high-performance capillary electrophoresis and methylation-sensitive arbitrarily primed PCR. However, the most common technique used today remains the bisulfite conversion method. This technique involves treating methylated DNA with bisulfite, which converts unmethylated cytosines into uracil.



Methylated cytosines remain unchanged during the treatment. Once converted, the methylation profile of the DNA can be determined by PCR amplification followed by DNA sequencing.

All utilities (reagents and plastics), except Ethanol and Conductive filter tips (must be ordered separately from Invitek Molecular GmbH), required for preparation of DNA are provided by the **InviMag® Bisulfite Conversion Kit/ IG**.

THE PRODUCT IS INTENDED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

Product use limitation

The product is only for Bisulfite conversion of DNA in low salt buffers or water, any other contaminant may interfere with the process.

Differing the starting material or flow trace may lead to inoperability. Therefore, neither a warranty nor a 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.

European Community risk and safety phrases for the components of the **InviMag® Bisulfite Conversion Kit/ IG** to which they apply are listed below as follows:

Solution BS



warning

Solution D



H314;317;412,P280-305+351+338

H314 P280-305-351-338

H314: Causes severe skin burns and eye damage.

H317: May cause an allergic skin reaction.

H412: Harmful to aquatic life with long lasting effects.

P280: Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338: 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® Bisulfite Conversion Kit/ IG

The **InviMag® Bisulfite Conversion Kit/ IG** provides a fast and efficient way for Bisulphite conversion of high quality DNA. The procedures are suitable for use with DNA Eluate. Samples can be fresh or frozen.

| Starting material | Yield | Time for preparation |
|--|---|--|
| For optimal results, the amount of input DNA should be 500 pg to 5 µg (20-50 µl eluted DNA) if using low amounts, please think of dilution effects and use a low elution volume | depending on starting amount DNA Recovery >50% Conversion Efficiency >99% | about 180 min per bisulfite conversion run |

Yield and quality of the converted DNA is suitable for PCR and methylation analysis by the respective methods (Real Time PCR, Sequencing, High Throughput Sequencing, Digital PCR and more).

For further information please contact: phone +49 (0) 30 9489 2901, 2910 in Germany and from foreign countries phone +49 (0) 30 9489 2907 or ask your local distributor.

Principle and procedure

The **InviGene® Bisulfite Conversion Kit** procedure comprises following steps:

- Denaturation and Bisulfite Conversion
- Binding of the DNA to the magnetic beads and Desulfonation
- Washing the bead bound DNA and elimination of ethanol
- Elution of converted DNA

Denaturation and Bisulfite Conversion

Samples are denatured/ converted at elevated temperatures in the present of **Solution BS**.

Binding of the bisulfite converted DNA and desulfonation

After addition of **Lysis Buffer HL** and a mix of **MAP Solution B** and **SNAP Solution** (magnetic beads) to the converted Samples, the DNA is bound to the surface of the beads. A followed additional washing step removes residual contaminants in the presence of **Wash Buffer II**. The desulfonation will be performed by using **Solution D**.

Washing the bead bound DNA and elimination of ethanol

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

Elution

The bisulfite converted DNA is finally eluted in Elution Buffer and ready-to-use in different subsequent downstream applications.

Important points before starting a protocol

Immediately upon receipt of the product, inspect the product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify Invitex Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the Invitex Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (see page 7). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipette tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipette tips.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and attractive goggles.
- Discard gloves if they become contaminated.
- Do not combine components of different kits, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- This kit should only be used by trained personnel.

Equipment and reagents 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.invitek-molecular.com)

1. Ethanol (96-100%) *
2. Disposable gloves
3. Pipet with tips

*The **InviMag® Bisulfite Conversion Kit/ IG** is validated with **Ethanol** absolute, reag. ISO, reag. Ph. Eur., ≥99.8% (GC), liquid (clear, colorless) from **Sigma-Aldrich** (Order Nr.32205).

Storage of starting materials

Please read the instructions carefully and conduct the prepared procedure.

Sampling and storage

Storage of DNA

A working stock of DNA can be stored at 2 – 4°C for several weeks. For long term storage DNA should be stored at -20°C, but storing at – 20°C can cause shearing, particularly if the DNA is exposed to repeated freeze-thaw cycles.

Yield and quality of bisulfite converted DNA

The yield of converted DNA in the **InviMag® Bisulfite Conversion Kit/ IG** procedure from purified DNA depends on the content, the sample source, transport, storage, and age of samples.

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 HL, SNAP Solution/ IG, MAP Solution B/IG, Solution D; Solution BS and the **Elution Buffer** are ready-to-use.

8 x 12 extractions

Add 84 ml of 96-100% Ethanol to each bottle **Wash Buffer II** and mix thoroughly.
Fill 30 ml of 96-100% Ethanol into each empty bottle **Ethanol**.

Before each run:

Transfer the complete content of one Solution BS tube to a Solution BS IG tube. Avoid oxygen input by pipetting carefully.

IMPORTANT: Please keep in mind that Solution BS is only usable for 1 run only.

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. However we recommend to apply the supplied foils beforehand (and afterwards on the used wells) on the unused wells of the Incubation Plate A and the Working Plate B.

Scheme of the InviMag® Bisulfite Conversion Kit/ IG

| | |
|--|---|
| <p>Add the samples (20-50µl) into the first free lane of the Incubation plate Add the Buffers in the Buffer loading rack.</p> | |
| | <p>sample is mixed with 150µl Solution BS</p> <p>Incubation at elevated temperature is performed for 60 min.</p> <p>30µl SNAP solution and 10µl MAP Solution B are added to the incubation plate.</p> <p>1 ml Lysis Buffer HL is added to the incubation plate.</p> <p>DNA binds to magnetic particles</p> <p>Magnetic separation</p> <p>Washing and desulfonation of the particle fixed DNA</p> <p>Magnetic separation</p> <p>Elution of converted DNA in 70 µl heated Elution Buffer</p> <p>Magnetic separation and removal of magnetic Beads</p> <p>Transfer of 50µl DNA in Elution plate</p> |

Preparing the samples for processing on the InviGenius® PLUS

Avoid thawing samples more than once.

The assay “DBISU_E50S50_MT” excludes the sample transfer.

General overview of the InviGenius® PLUS

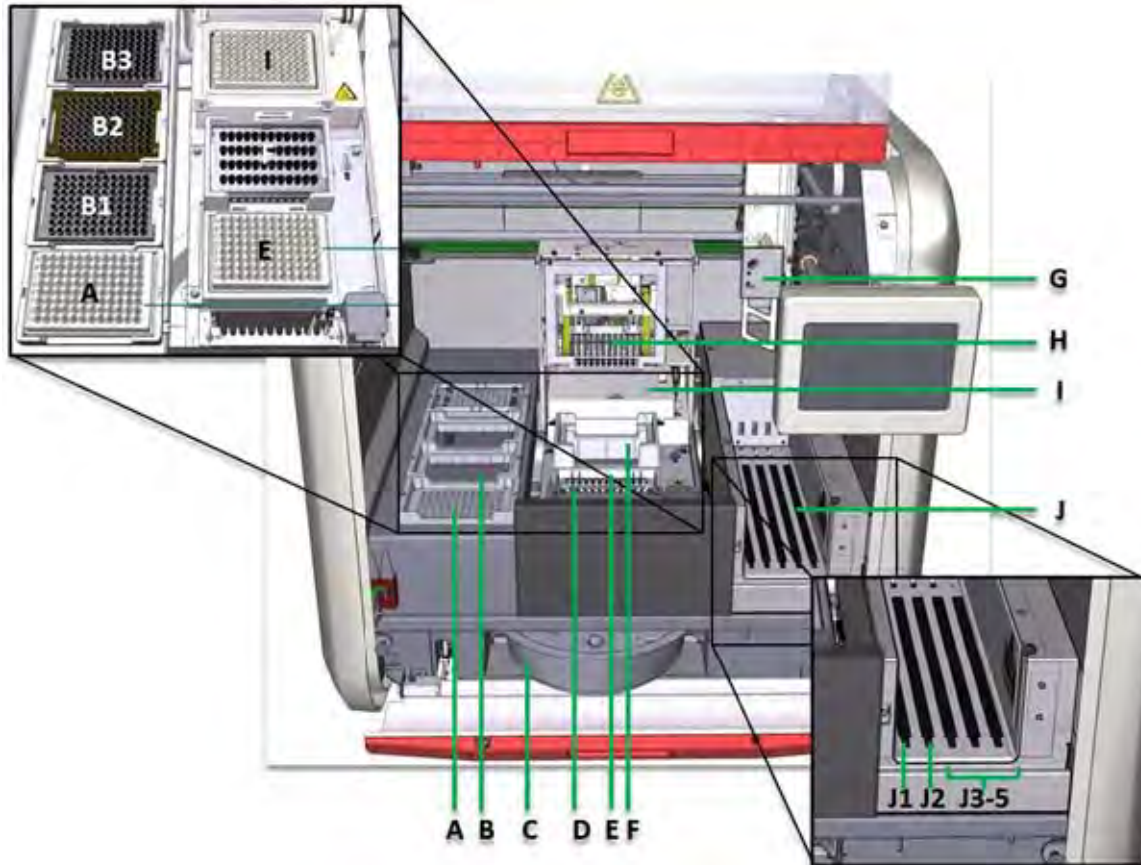


Figure 1: Frontal view of the InviGenius® PLUS system

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 Invitex 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

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).

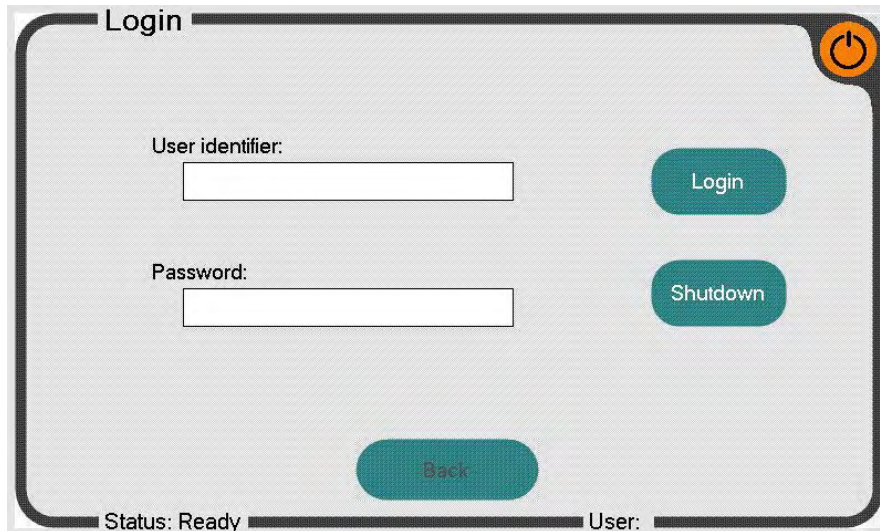


Figure 2: Login screen of the InviGenius® software

Log in with the provided user name and password.

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

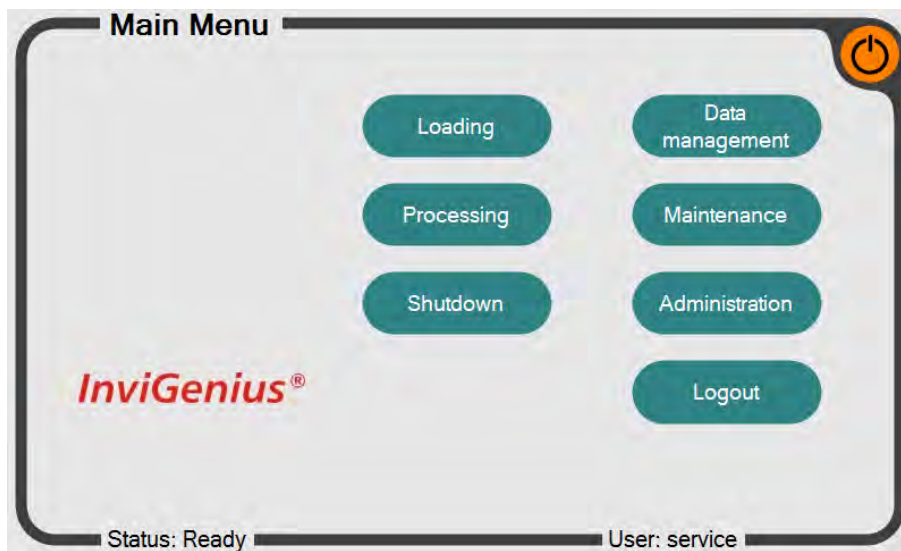


Figure 3: Main menu of the InviGenius® software

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).

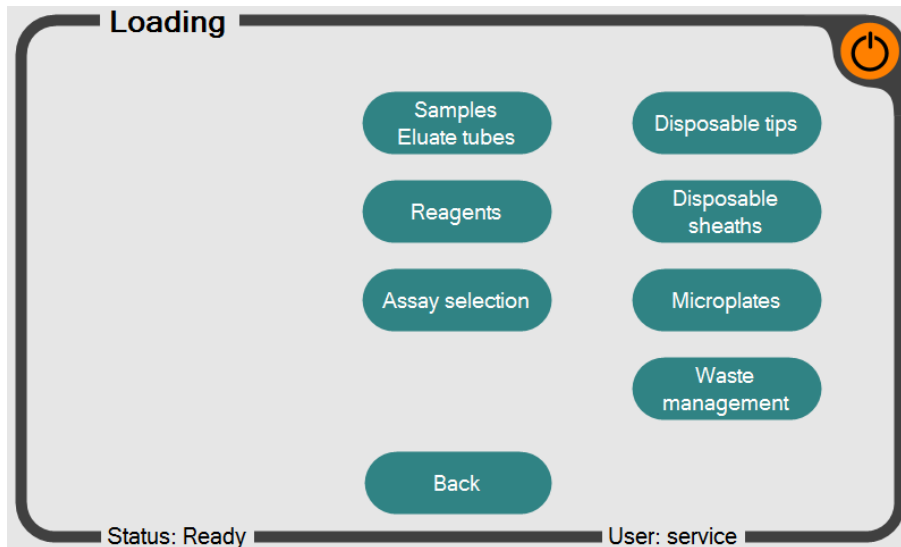


Figure 4: Loading screen of the InviGenius® software

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

The Sample loading screen appears (Figure 5).

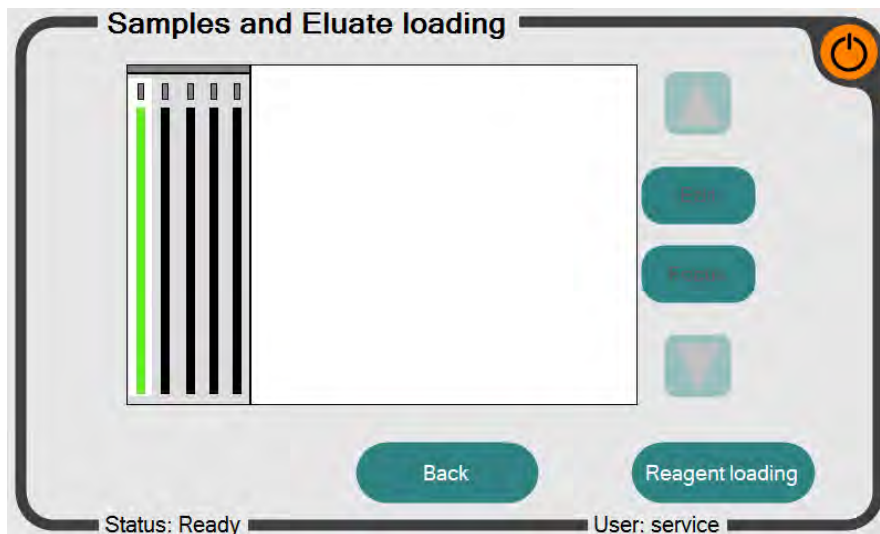


Figure 5: “Sample and Eluate loading” screen of the InviGenius® software

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

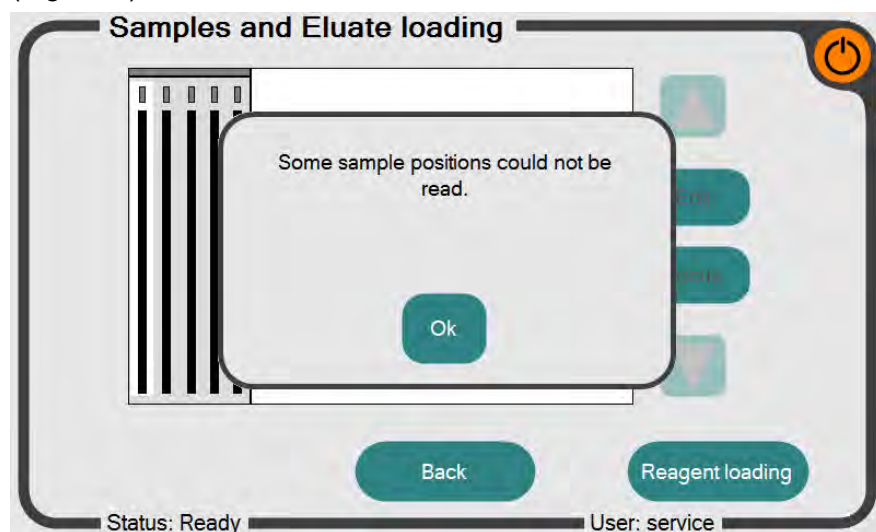


Figure 6: “Sample and Eluate loading” screen of the InviGenius® software

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

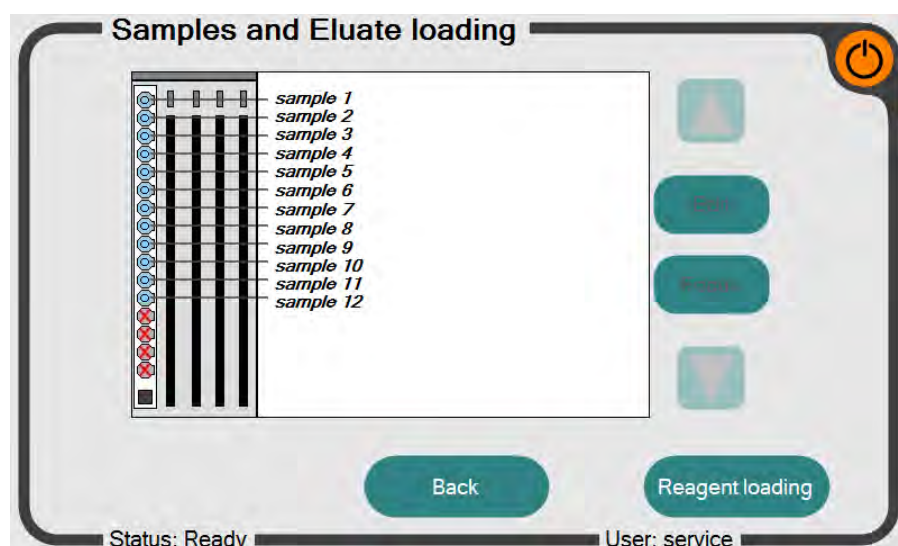


Figure 7: “Sample and Eluate loading” screen of the InviGenius® software with 12 named samples

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; 2 ml Cryotubes from Invitex or 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).

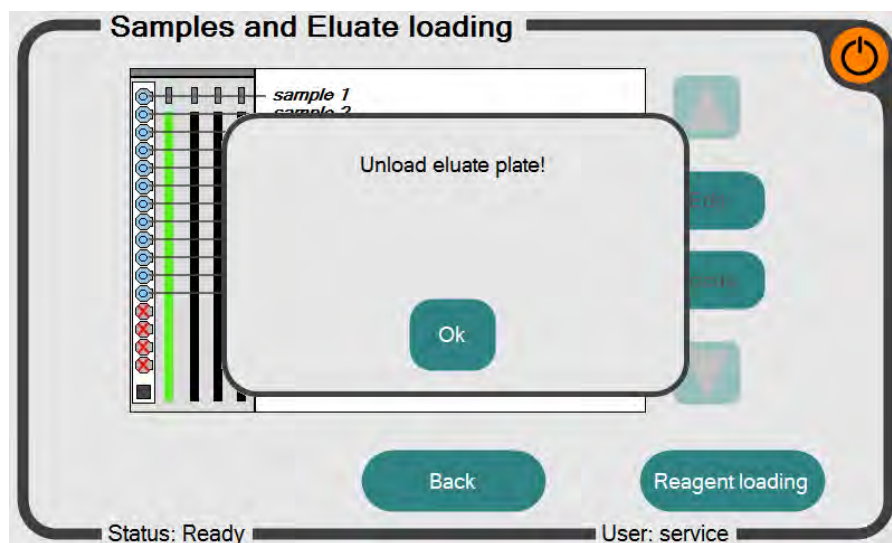


Figure 8: Screen of the InviGenius® software to unload eluate plate

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

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

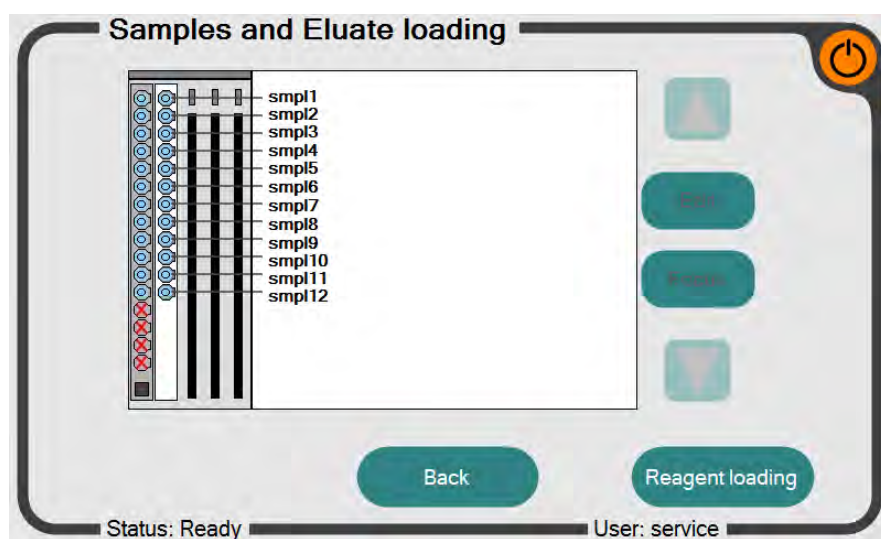


Figure 9: “Sample and Eluate loading” screen of the InviGenius® software

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.

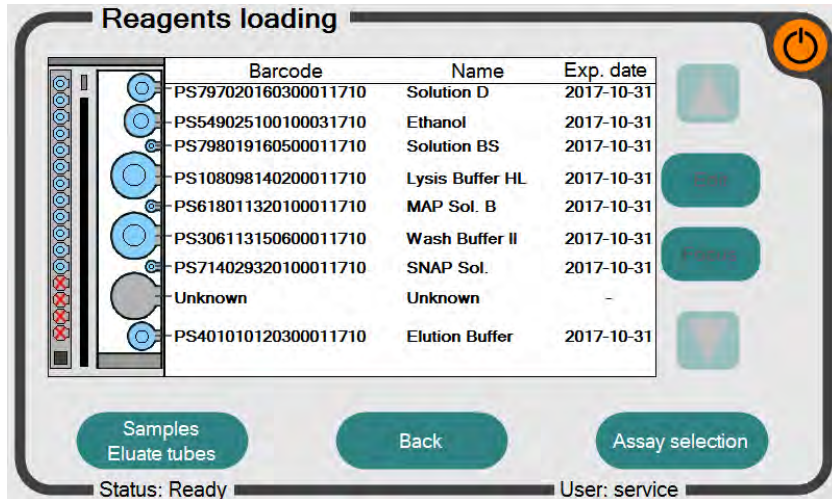


Figure 10: “Reagent loading” screen of the InviGenius® software

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 “DBISU_E50S50_MT” (see also chapter “Preparing the samples for processing on the InviGenius®-system”) and proceed with disposable tip loading.

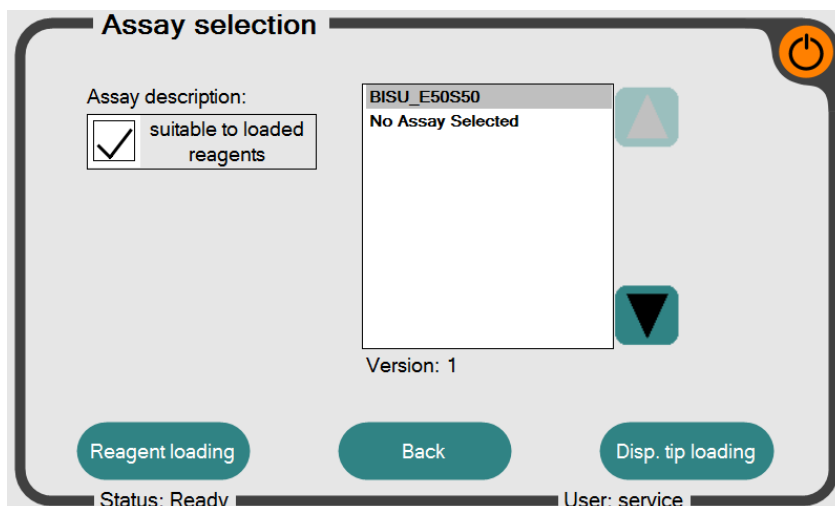


Figure 11: “Assay-selection” screen of the InviGenius® software

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”.”

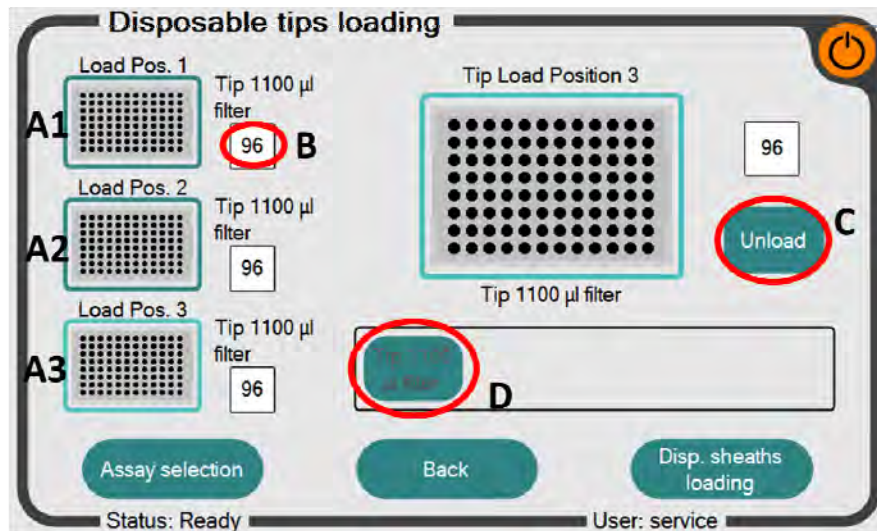


Figure 12: Disposable tip loading screen of the InviGenius® software

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® Bisulfite Conversion 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. Invitex 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 Invitex Molecular. Invitex 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.

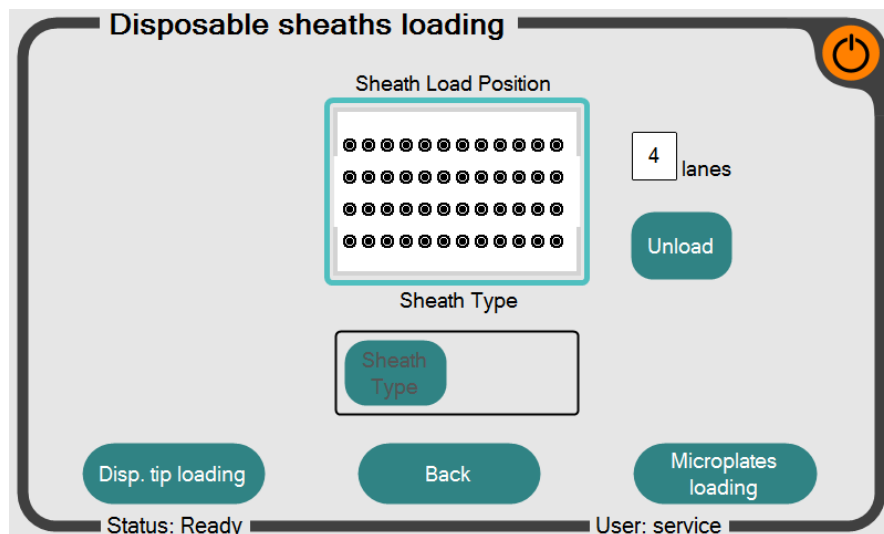


Figure 13: Disposable sheaths loading screen of the InviGenius® software

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 amount 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 Invitex 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 A, Working Plate B and Elution Plate E (if no eluate rack) are loaded within the plate loading screen (Figure 14).

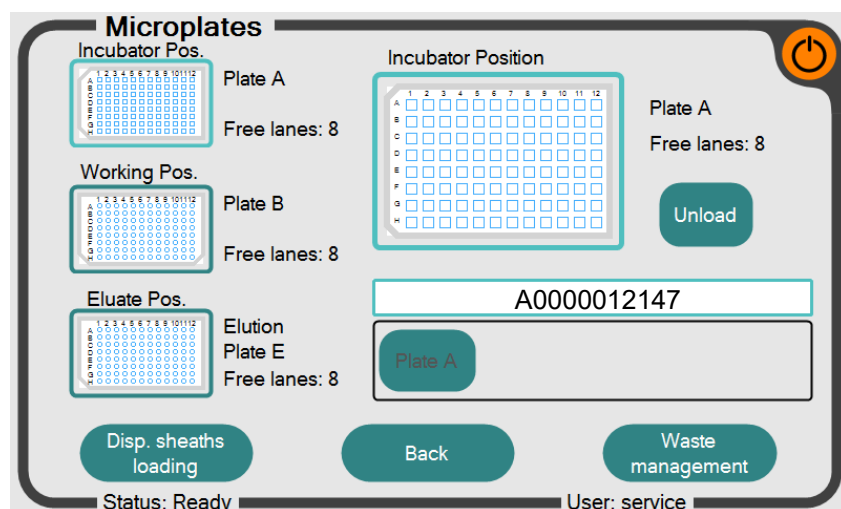


Figure 14: Plate loading screen of the InviGenius® software

In general, the Incubation Plate A 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 14 Incubation plate A 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 working position and one free lane in the eluate position or an eluate rack.

You can use the incubator Plate as Sample Plate for 8 Runs. Therefore you have to register the incubation Plate B by the barcode in every run. The system will also register for this position the used Sample Lanes per run. For further experiments you can use the same incubator Plate with the remaining free Lanes

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.

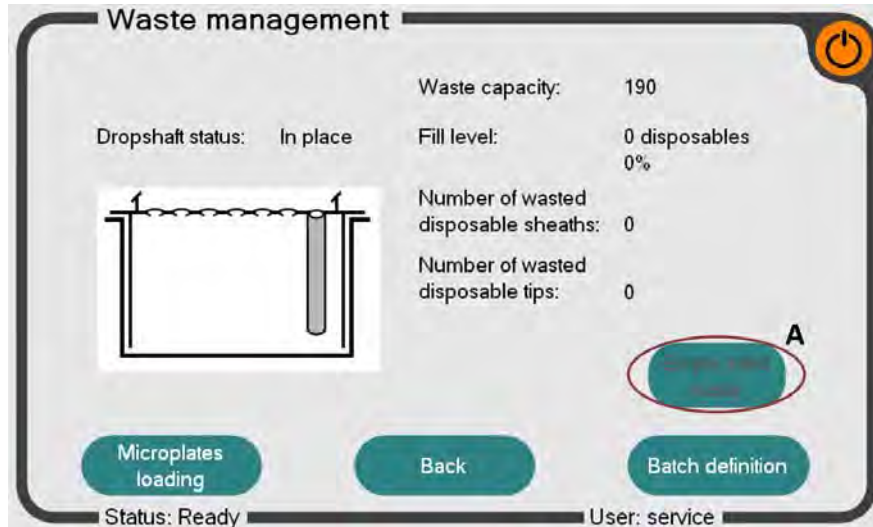


Figure 15: Waste-management-screen of the InviGenius® software

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.

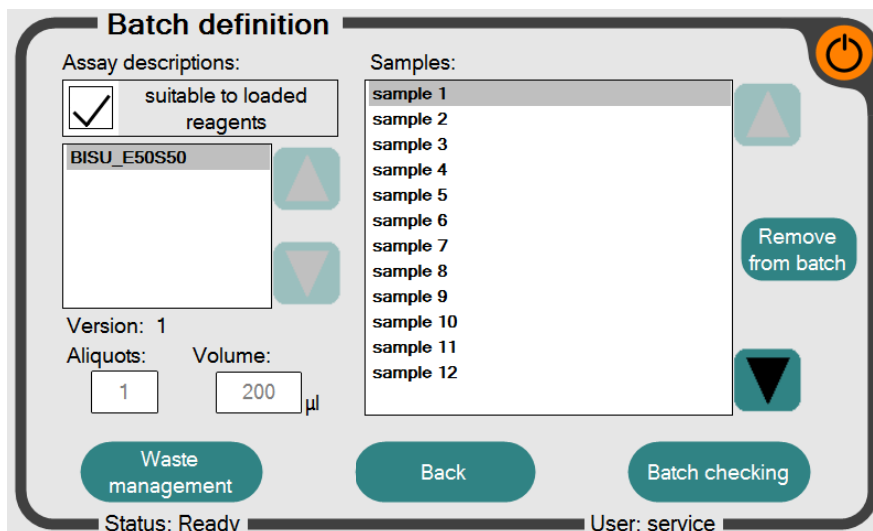


Figure 16: Batch-definition-screen of the InviGenius® software

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).

Batch checking:

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

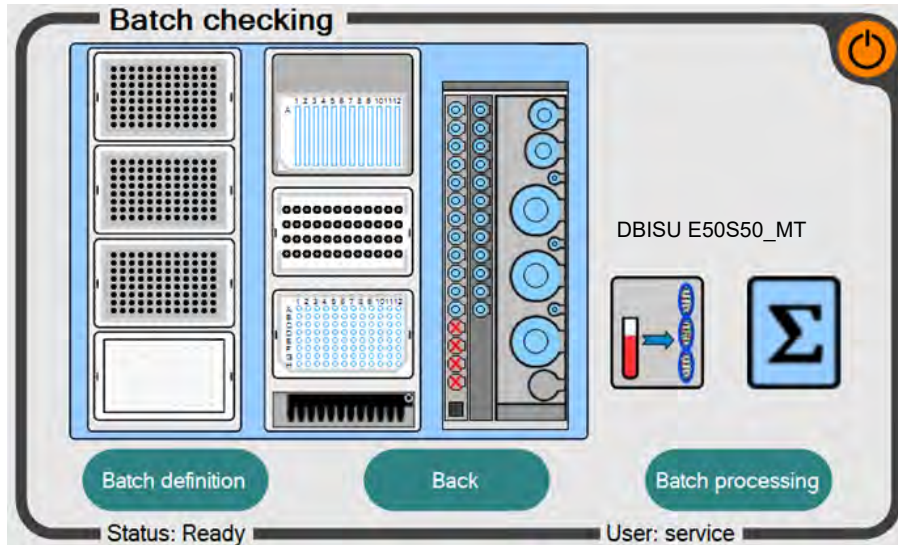


Figure 17: Batch-definition-screen of the InviGenius® software

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”.

Batch processing:

Press the bottom “Batch processing”.

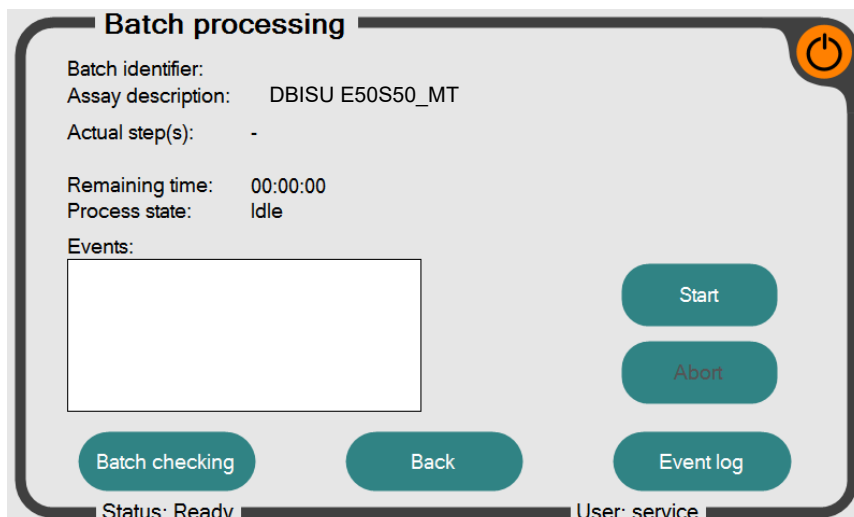


Figure 18: Batch-processing-screen of the InviGenius® software

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

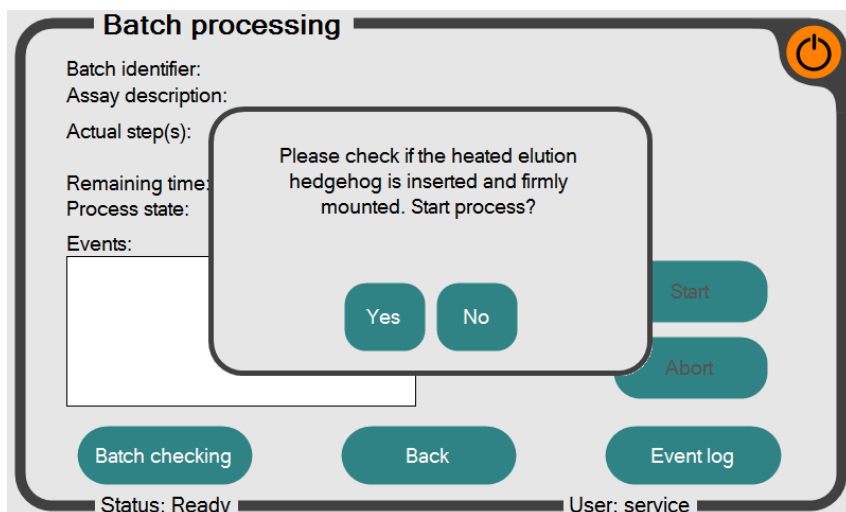


Figure 19: last check-screen of the InviGenius® software

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.

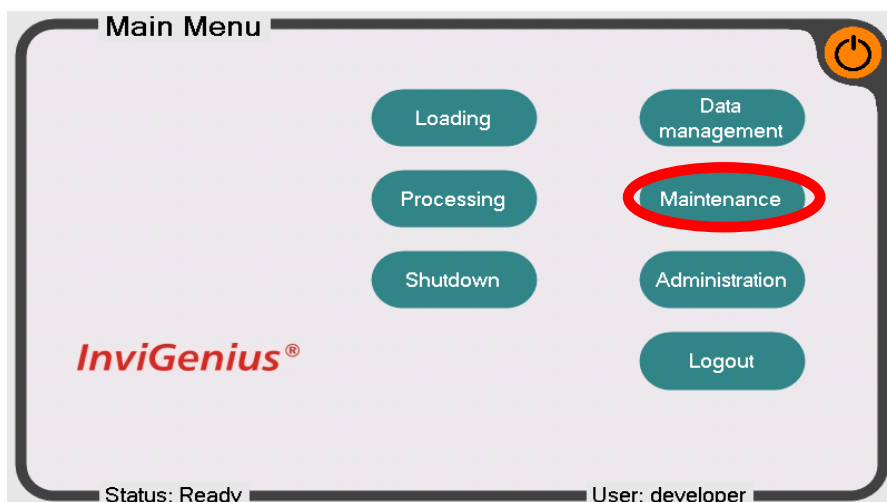


Figure 19: Main screen of the InviGenius® software

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

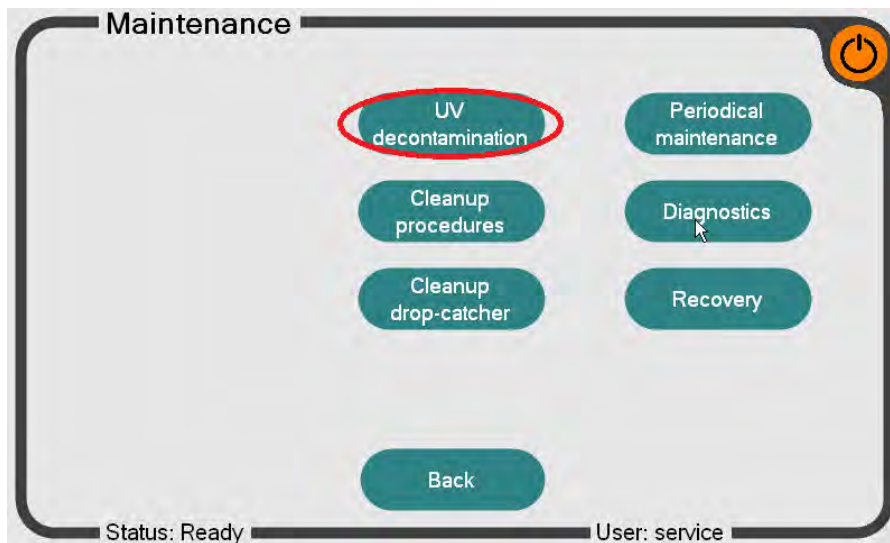


Figure 20: Maintenance screen of the InviGenius® software

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.

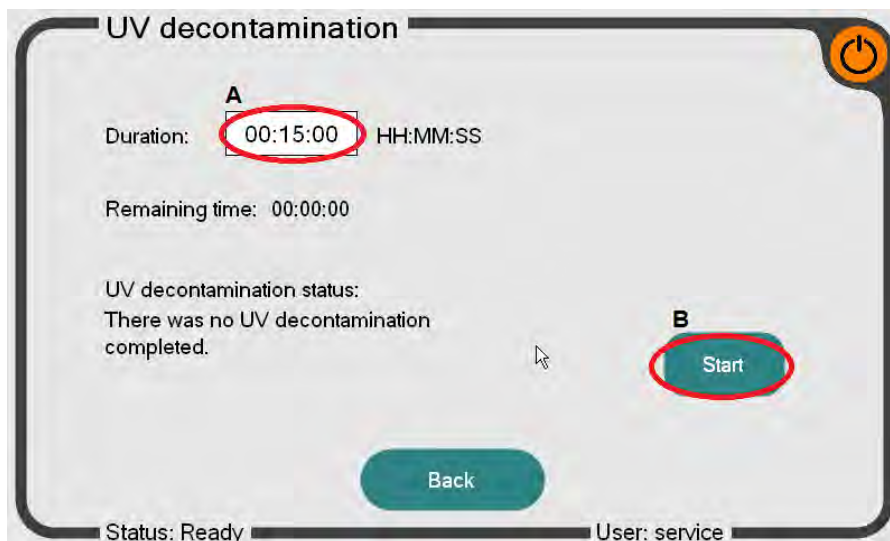


Figure 21: UV decontamination screen

When the decontamination process 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.

Troubleshooting

| Problem | Probable cause | Comments and suggestions |
|--------------------------------------|---|---|
| Pipetting distribution errors | Reagent / buffer transfer failed / incomplete | Ensure that the Wash Buffer II and Ethanol are filled up properly with ethanol Ensure that the Solution BS is completely transferred to the 3.5 ml Receiver Tube (2 ml) Do not reuse bottles more often than described |
| Low conversion rate | Too much volume of your starting material | Ensure that your starting Volume is in a range of 20-50 μ l (think of the dilution effect) |
| DNA fragments too short | the concentration of your sample is too low | Ensure that the storage conditions of the starting material was correct Avoid multiple freezing and thawing cycles of the sample Ensure that your DNA is concentrated in a range of 500 pg to 5 μ g |
| | Combination of reagents from different kits or blocked barcode during reagent loading procedure | 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 |
| No assay selectable | 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 |

Ordering information

| Product | Package size | Catalogue No. |
|---------------------------------------|--------------|---------------|
| InviMag® Bisulfite Conversion Kit/ IG | 8 x 12 preps | 3030200100 |

InviGenius® and consumables

| | | |
|---|----------------|------------|
| InviGenius® PLUS | 1 unit | 5011102000 |
| 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 | | |
| Conductive filter tips, 1 ml | 10 x 96 pieces | 5011100400 |
| Waste tray/ IG | 25 pieces | 5011100100 |
| Sheath Bundle | 10 x 48 pieces | 5011100300 |
| Sheaths | 1000 pieces | 5011100200 |

INVITEK
Molecular

Invitek Molecular GmbH
Röbert-Rössle-Str. 10
13125 Berlin

Phone: +49 30 94 89 29 01
Fax: +49 30 94 89 29 09
info@invitek-molecular.com

www.invitek-molecular.com

0063030200 V-01-2020