Instruction for InviMag® Blood DNA Mini Kit /IG

The **InviMag® Blood DNA Mini Kit/ IG** combines the advantages of the innovative Invisorb® technology with easy handling of magnetic particles in combination with the InviGenius® robotic platform for a very efficient and reliable isolation of nucleic acids with a high purity.

The **InviMag® Blood DNA Mini Kit/ IG** is the ideal tool for a walk-away automated isolation and purification of highly pure total (genomic and mitochondrial) DNA from 200 µl whole blood samples (EDTA or citrate stabilized *but not heparine*). The kit has been designed for an optimal use on the InviGenius® workstation. The interplay of the DNA extraction and purification chemistry provided by the **InviMag® Blood DNA Mini Kit/ IG** was intensely tested and validated.

The DNA-binding magnetic particles are characterized by a high surface area, an uniform size distribution, and a good suspension stability and are therefore highly suitable for high throughput processing.

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

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

The kit is neither validated for the isolation of genomic DNA from cultured or isolated cells, from tissue, blood cards, dried blood stains, urine. The application of the kit for isolation and purification of viral DNA has not been evaluated.

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

Contents

Kit contents of InviMag® Blood Mini Kit /IG

Store the **MAP Solution B** at 2 - 8 °C!
Store lyophilized **Proteinase K** at 2 - 8 °C!
Store dissolved **Proteinase K** at –20 °C!
Store all other kit components at room temperature (RT)!

Component	8 x 12 preps	reagent sufficient for
Catalog No	2431120100	
Lysis Buffer A	30 ml	8 runs
Proteinase K / IG	8 x 800 µl	1 vial per run
MAP Solution B/ IG	8 x 800 µl	1 vial per run
Binding Buffer B6	60 ml	8 runs
Wash Buffer I	50 ml final volume 100 ml	8 runs per bottle
Wash Buffer II	2 x 30 ml final volume 2 x 100 ml	4 runs per bottle
Elution Buffer M	30 ml	8 runs per bottle
Incubation Plate A	1	8 runs per plate
Working Plate A	4	2 runs per plate
Elution Plate C	1	8 runs per plate
Sheath Box	1	4 runs per plate
Sealing Foils	4	
Incubator Stripe Foils *)	1	

Initial steps:

Resuspend lyophillized **Proteinase K** by addition of 800 µl dd-H_2O,to each vial mix thoroughly and store like described below!

Add 50 ml of 96-100% ethanol to the bottle **Wash Buffer I.** Mix thoroughly and always keep the bottle firmly closed!

Add 70 ml of 96-100% ethanol to the bottle **Wash Buffer II.** Mix thoroughly and always keep the bottle firmly closed!

*) Use the **Incubator stripe foil** to seal unused of the **Incubation Plate A**.

Symbols

 manufacturer

 lot number

 catalogue number

 date of manufacture

 expiry date

 consult operating instructions

 temperature limitation

 do not reuse

Storage

All buffers and kit contents of the **InviMag® Blood Mini Kit/ IG**, except **Proteinase K** and **MAP Solution B** (magnetic beads) should be stored at room temperature and are stable for at least 12 months under these conditions.

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

Proteinase K: Lyophilized Proteinase K should be stored at 4-8°C. Dissolved Proteinase K must be stored at -20°C. Dividing the Proteinase K into aliquots and storage at –20°C is recommended.

MAP Solution B: The magnetic bead solution should be stored at 4-8°C.

Wash Buffers: Wash Buffers charged with ethanol should be stored at room temperature and should be appropriately sealed. If there are any precipitates within the provided solutions solve these precipitates by careful warming up to room temperature (up to 30°C)

Quality control

STRATEC Molecular guarantees the correct function of the **InviMag® Blood DNA Mini Kit/ IG** for applications as described in the manual. In accordance with STRATEC Molecular's certified QM system each component of the **InviMag® Blood DNA Mini Kit/ IG** was tested against predetermined specifications to ensure consistent product quality.

All products sold by STRATEC Molecular are subjected to extensive quality control procedures according to ISO 9001-2000 and are warranted to perform as described when used correctly. Any problems should be reported immediately.

If you have any questions or problems regarding any aspects of **InviMag® Blood DNA Mini Kit/ IG** or other STRATEC Molecular products, please do not hesitate to contact us.

For technical support or further information please contact:
from Germany: +49-(0)30-9489-2901/ 2910
from abroad: +49-(0)30-9489-2907
or contact your local distributor.

Intended use

The **InviMag® Blood Mini Kit /IG** has been designed for fully automated extraction and purification of genomic DNA from 200 µl whole blood from 1 – 12 whole blood samples using magnetic beads and the InviGenius® instrument.

The nucleic acid isolation protocol is suitable for routinely walk away automated preparation of DNA from fresh or frozen blood sample. For reproducible and high yields an appropriate sample storage is essential (see “Sampling and storage of the starting material”, page 8).

Common blood collection tubes (not provided) and anticoagulants (EDTA and citrate, *but not heparin*) can be used to gather a set of blood samples. All utilities (reagents and plastic ware, beside the filter tips) necessary for preparation of total DNA from blood are provided by the **InviMag® Blood Mini Kit /IG.**

The procedure of the **InviMag® Blood Mini Kit / IG** has been optimized for the isolation of genomic DNA from up to 200 µl starting material. However, we advise to use at least a sample volume of 1000 µl per 12 - 17 mm tube to prevent pipetting distribution errors. The final processed sample volume is 200 µl.

The product is intended for use by professionals such as technicians, physicians and biologists trained in molecular biological techniques. It can be used with any downstream application, employing enzymatic amplification or other modifications of DNA followed by signal detection or amplification. Any diagnostic results generated using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings. To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

Product use limitation

The kit is validated for the use of blood samples only. The isolation of DNA from other sources like stool samples, tissues, bacteria, fungi or viruses was neither tested nor validated. No guarantee in operability is issued with differing starting materials, sample type or change in the procedure.

All kit components are not intended for consumption.

All products sold by STRATEC Molecular are subjected to extensive quality control procedures (according to EN ISO 9001-2000 or EN ISO 13485-2003) and are warranted to perform as described when used correctly. Any problems should be reported immediately.

The chemicals and plastic parts are for laboratory use only. They have to be stored in the laboratory and have not to be used for other purposes than intended.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. Carefully heed the legal requirements for working with biological materials.

If buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any possible injuries.

STRATEC Molecular has not tested the waste generated by the **InviMag® Blood Mini Kit /IG** procedures for residual infectious materials. Contamination of the waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, the waste has to be considered infectious and should be handled and discarded according to local safety regulations.

Below European Community risk and safety phrases for the components of the **InviMag® Blood Mini Kit /IG** to which they apply, are listed.

Wash Buffer I contains guanidine thiocyanate which is an irritant.

Lysis Buffer A

danger
H-319 P305-351-338

Binding Buffer B6

danger
H319-338 P210-233-305-351-338

Proteinase K

danger
H315-319-334-335 P280-305-351-338-310-405

Wash Buffer I

warning
H302-312-332-412 EUH032 P273

H319:	Causes serious eye irritation.
H225:	Highly flammable liquid and vapour.
H336:	May cause drowsiness or dizziness.
H315:	Causes skin irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
H302:	Harmful if swallowed.
H312:	Harmful in contact with skin.
H332:	Harmful if inhaled.
H412:	Harmful to aquatic life with long lasting effects.
EUH032:	Contact with acids liberates very toxic gas.
P305+P351+P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P210:	Keep away from heat/sparks/open flames/hot surfaces. — No smoking.
P233:	Keep container tightly closed.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.
P273:	Avoid release to the environment.#

Emergency medical information in english and german language can be obtained 24 hours a day from:

Poison Information Center Freiburg, Germany: Tel.: ++49 761-19240

Product characteristics of the InviMag® Blood DNA Mini Kit /IG

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

Starting Material	Typical Yield	Time for preparation	Ratio
1-200 µl fresh or frozen whole blood	2 - 10 µg, depends on the blood sample (storage and source)	about 60 min per run (12 samples)	A_{260}:A_{280}: 1.6-2.0

The DNA isolation process is based on the interaction of nucleic acids with silica coated magnetic particles under adapted buffer conditions. The **InviGenius®** 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®** instrument uses magnetic rods to transport the DNA-binding magnetic particles through the various purification phases: Binding-Washing-Elution. The volume of buffers and other liquids necessary for DNA isolation is reduced to a minimum. Eliminating the direct liquid handling and increasing the automation level results in a fast, reliable and robust technique. The overall efficiency speeds up the procedure. The process on **InviGenius®** is total in- process control – including e.g. completely reagent tracking „on the bottle“ – lot, shelf live, amount, a detailed inventory checks helps to eliminate human error, a data storage, backup and archiving. The workflow is from sample to ready to use DNA.

Due to the high purity, the eluted total (genomic and mitochondrial) DNA is ready to use for a broad panel of downstream applications:

- PCR*, Reverse transkriptase PCR, qPCR
- Restriction Enzyme Digestion
- HLA Typing
- Southern Blot.

For the isolation of DNA from a single blood sample STRATEC Molecular offers the **Invisorb® Spin Blood Mini Kit** or for 8 – 96 samples the **Invisorb® DNA Blood Mini HTS 96 Kits** for use on a centrifuge, vacuum manifold or robotic station.

For further information please contact: phone.: +49 (0) 30 9489 2894, 2910 in Germany and from foreign countries phone.: +49 (0) 30 9489 2907 or your local distributor.

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

Sampling and storage of starting material

For reproducible and high yields an 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.

Blood:
Best results are obtained using fresh blood samples. Mammalian blood samples (stabilized with EDTA or citrate but **not** heparin) can be stored at room temperature (18-25°C) for 2-3 hours. For short time storage (up to 24 h) samples should be stored at 4-8°C. For long term storage, we recommend to freeze the samples at -20°C or -80°C. Avoid multiple thawing and freezing cycles of the sample(s) before isolating the DNA. Various different primary tubes, blood collection system (e.g. Sarstedt, Greiner) and anticoagulants (except heparin) can be used to collect blood samples for the **InviMag® Blood DNA Mini Kit /IG** procedure

STRATEC Molecular will not take responsibility if other kind of samples are used than blood or if the prepared procedure is modified.

Principle and procedure

The **InviMag® Blood DNA Mini Kit/ IG** procedure comprises following steps:

- lysis of blood cells and protein digestion
- binding the genomic DNA to the magnetic beads
- washing the bead bound DNA and elimination of ethanol
- elution of genomic DNA.

After lysis the DNA binds to the magnetic beads, contaminations and enzyme inhibitors are efficiently removed during the following four wash steps and highly purified DNA is eluted in Elution Buffer or water.

Lysis
Samples are lysed under non chaotropic conditions in a 2.0 ml Deep Well Plate at elevated temperatures in the presence of 200 µl **Lysis Buffer A** and 40 µl **Proteinase K**.

Binding of the genomic DNA
After addition of 400 µl **Binding Buffer B6** and 40 µl **MAP Solution B (magnetic beads)** to the lysate, the DNA is bound to the surface of the beads.

Removing residual contaminants
Contaminants are efficiently washed away using 800 µl **Wash Buffer I** and **II**, respectively, while the DNA remains bound to the magnetic beads.

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

The blood assays stored in the instrument memory contains two different elution volumes / protocols.

The user can choose between

DBLD_E100_S200 (DNA from Blood; E=Elution 100µl; S=Sample 200µl)

DBLD_E200_S200 (DNA from Blood; E=Elution 200µl; S=Sample 200µl)

50µl will remain in the elution lane with any rests of beads.

Yield and quality of genomic DNA

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

Typically, a volume of 200 µl of a whole blood sample from a healthy individual with an elevated white blood cell content - ranging from $3x10^6$ to $1x10^7$ cells/ml - will yield at least 3 µg of genomic DNA. The typical yield usually expected from the **InviMag® Blood DNA Mini Kit /IG** is about 2-10 µg DNA. If a whole blood sample is mixed with anticoagulant containing buffer solutions the overall leukocyte concentration decreases and the yield of the DNA extraction procedure is reduced.

Important notes

Important points before starting a protocol

After receiving the kit, check the kit components for damage. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety information" (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 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.
- 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 re-dissolve any precipitates by incubation at 30°C. Swirl gently to avoid foaming.
Lysis Buffer A, Binding Buffer B6 and **Elution Buffer M** are ready to use.

8 x 12 DNA-extractions:
Resuspend lyophillized **Proteinase K** by addition of 800 µl dd-H_2O, mix thoroughly and store diluted **Proteinase K** at –20°C.
Add 50 ml of 96-100% ethanol to the bottle **Wash Buffer I**. Mix thoroughly and always keep the bottle firmly closed!
Add 70 ml of 96-100% ethanol to the bottle **Wash Buffer II**. Mix thoroughly and always keep the bottle firmly closed!

Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Conductive pipette tips (see ordering information, page 26)
- Disposable gloves
- PBS buffer
- ddH_2O
- Vortexer
- 96-100% ethanol
- primary tubes (e.g. see below)

Possible primary tubes, manufacturer, cat. no.

Venosafe, 5,5ml, Ref, VF-076SDK, Terumo
Vacuette, 2ml Ref, A110500I, Greiner bio-one
Vacuette, 9ml, Ref, 455036, Greiner bio-one
BD Vacutainer, 2,7 ml, Ref, 363048
BD Vacutainer, 6 ml, Ref, 367864
BD Vacutainer, 10 ml, Ref, 367525
BD Vacutainer 5,0 ml, Re
Sarstedt Monovette, 8,5m
PS Tube Sarstedt 5ml, Ref : 55.476
Sarstedt Monovette 4,5 m
Sarstedt Monovette 7,5 m
Sarstedt Monovette 9,0 ml

Important indications

1. Minimum volume of samples in primary tubes

The procedure of the **InviMag® Blood Mini Kit /IG** has been optimized for the isolation of genomic DNA from up to 200 µl whole blood. We advise to provide at least 1000 µl blood per sample tube to prevent pipetting distribution errors during processing. ´

2. Sample volume smaller than 200 µl

For samples of a smaller volume than 200 µl please fill the blood tube with PBS to a volume of minimal 1000 µl.

3. Elution volume

The final processed sample volume is 200 µl. The procedure also offers a variation of the elution volume by offering two assays with 100 µl or 200 µl elution volume in respect to the user´s demand. Typically, 200 µl eluate results in app. 10-50 ng DNA / µl, whereas 100 µl eluate results in app. 20-100 ng DNA / µl.

The blood assays stored in the instrument memory contains two different elution volumes / protocols.

The user can choose between:

DBLD_E100_S200 (DNA from Blood; E = Elution volume 100 µl; S = Sample volume 200 µl)

DBLD_E200_S200 (DNA from Blood; E = Elution volume 200 µl; S = Sample volume 200 µl)

Important: *DBLD_E100_S200 assay should only be used if whole blood with a very low content of leukocytes will be used. Using "normal" whole blood will very likely result in an incomplete elution due to the high concentration of leukocytes present in "normal" blood and will most probably lead to a carry over of magnetic beads.*

Prevention of Cross-contamination

To comply with the demanding guidelines of *in-vitro*-Diagnostics we programmed the InviGenius® to route the pipettor in such a way that possible contamination-risks are minimized. However we recommend to apply the supplied well-strips and –foils after a run on the used wells of the Incubation Plate A and the Working-Plate B.

Note: *Don't forget to seal the used wells with the provided sealing foils of the Working Plate A before starting a run.*

General overview of the InviGenius® system

Figure 3: Frontal view of the InviGenius® system

There are three rack positions on the InviGenius® system which can be loaded with the corresponding plates: the incubation rack (A), the working rack (B), and the elution rack (C).

The lysis is performed in the Incubation plate (A), whereas the washing and elution process is performed in the working plate (B). The eluate - containing the extracted nucleic acids – will be finally transferred to the Elution Plate C.

Additionally, there are three loading racks for disposable tip trays (D1-D3) and one rack position (E) for the disposable sheaths. The loading bay (F) is located at the very right side of the instrument. The sample rack is loaded into the far left lane whereas the reagent rack is located at the right of the loading bay.

The Magnetic Separation Head (MSH) (G) is located on top of the incubator lid (parking position). The fully automatic pipettor (H) is installed above the loading bay (parking position). The disposable waste tray (I) is located behind the lower cover of the InviGenius®.

Interaction with the InviGenius® instrument is done by use of the touch LCD (J) located at the top front right side.

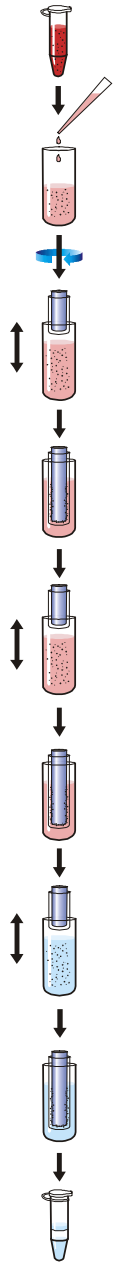
Scheme of the InviMag® Blood DNA Mini Kit/ IG

Add the Buffers in the Buffer loading rack

Add the primary tubes in the sample loading rack

Attention please: *Minimum sample volume per vial: 1000 µl*

Select the Elution volume by using the relevant run file (see important indication, page 10



200 µl sample will be mixed with 200 µl **Lysis Buffer A** and **40 µl Proteinase K** and incubated at 56°C for 10 min

400 µl **Binding Buffer B6** and 40 µl **MAP Solution B** will be added to the lysate

DNA binds to magnetic particles

magnetic separation

washing of the particle fixed genomic DNA
1 x 800 µl **Wash Buffer I**
1 x 950 µl **Wash Buffer II**
1 x 700 µl **Wash Buffer II**

magnetic separation

elution of genomic DNA in 100 µl / 200 µl **Elution Buffer M**

magnetic separation
removal of **MAP Solution B/ IG**

transfer of 50 / 100 µl pure DNA to the final position

Preparing the samples for processing on the InviGenius®

Please read the instructions carefully and conduct the prepared procedure.

Important Note: *The protocol has been optimized for the isolation of genomic DNA from up to 200 µl of whole Blood /EDTA, Citrate stabilized).*
To prevent possible distribution errors it is highly recommend to use at least 1000 µl of sample in total to ensure stable processing.

Attention: *Before you start, dissolve one vial of Proteinase K/ IG with 800 µl distilled water.*

Reconstitute the Wash Buffer

1. Extraction of genomic DNA from whole blood

This type of sample can be processed directly without any preparations. Please make sure to supply at least 1000 µl or dilute with PBS up to this volume.

DBLD_E100_S200 (DNA from Blood; E = Elution volume 100 µl; S = Sample volume 200 µl) (processed sample volume is 200 µl, elution volume 100 µl)

DBLD_E200_S200 (DNA from Blood; E = Elution volume 200 µl; S = Sample volume 200 µl) (processed sample volume is 200 µl, elution volume 200 µl)

2. Extraction of genomic DNA from swab samples

Rinse each swab with 1000 µl cooled water or cooled PBS in a primary tube. Remove the swab after sqeezing and load it into the InviGenius® .

DBLD_E100_S200 (DNA from Blood; E = Elution volume 100 µl; S = Sample volume 200 µl) (processed sample volume is 200 µl, elution volume 100 µl)

2. Extraction of genomic DNA from buffy coat

Maximum 250 µl buffy coat can be used. Please make sure that this samples is diluted with PBS and supply at least 1000 µl.

DBLD_E200_S200 (DNA from Blood; E = Elution volume 200 µl; S = Sample volume 200 µl) (processed sample volume of diluted sample is 200 µl, elution volume 200 µl)

Prevention of cross-contamination

To comply with the demanding guidelines of *In-vitro*-Diagnostics we programmed the InviGenius® to route the pipettor in such a way that possible contamination-risks are minimized. However we recommend to apply the supplied well-strips and –foils beforehand on the unused wells of the Incubation Plate A and the Working-Plate B.

To prevent any form of salt-crystallization of used lysis-broth it is recommended to reseal used wells after a run.

Preparing and loading of the InviGenius® system

Preparing the reagents:

Before you start, dissolve one vial of Proteinase K with 800 µl distilled water.

Preparing the system:

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

After successful initialization of the InviGenius® system a login screen appears (Figure 4). Login with the provided user name and password.

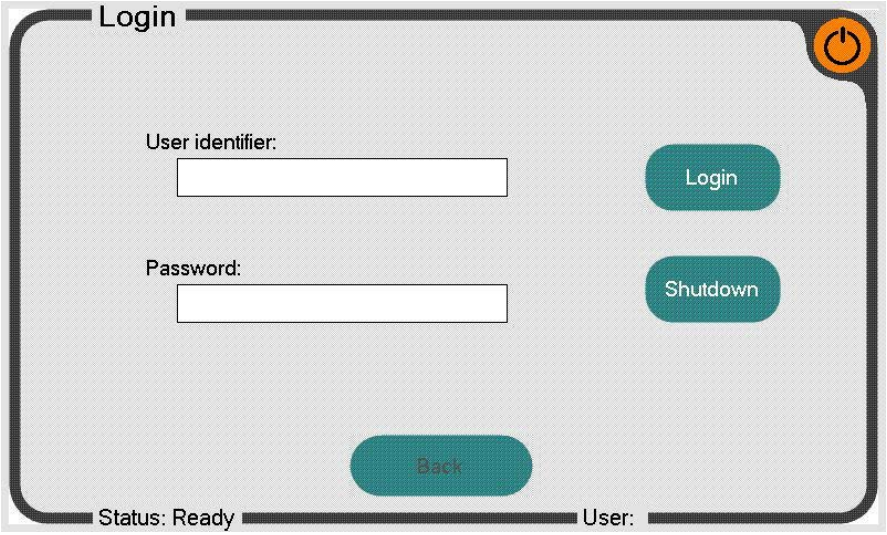


Figure 4: Login screen of the InviGenius® software

After logging in the main screen of the InviGenius® software appears (Figure 5). Select “Loading” to start loading the system and prepare for starting a run. Select “Processing” to define and run an assay if the system has been already loaded.

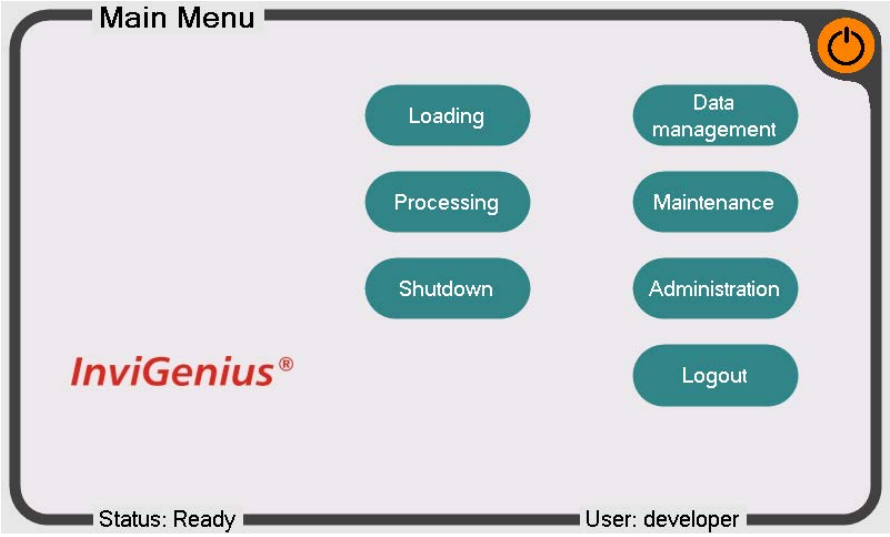


Figure 5: Main menu of the InviGenius® software

Sample Loading:

After selecting “Loading” the sample loading screen appears.

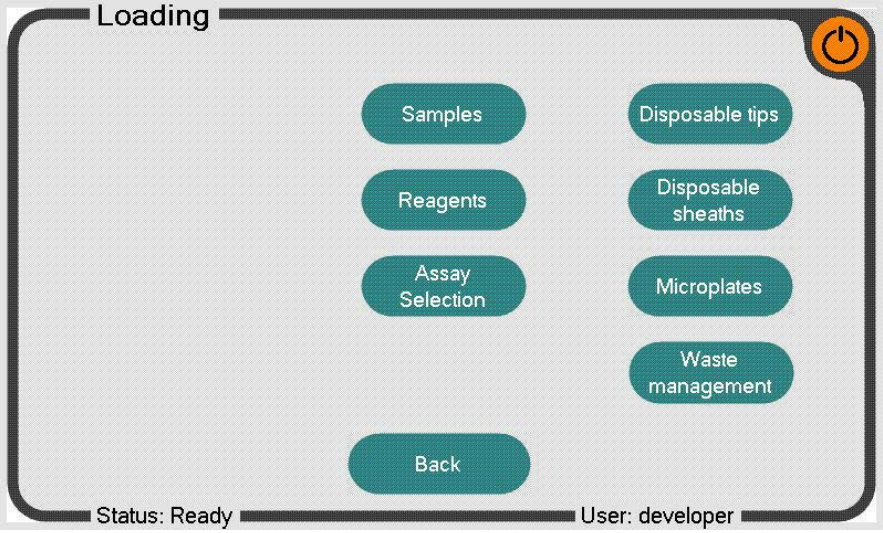


Figure 6: Loading screen of the InviGenius® software

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

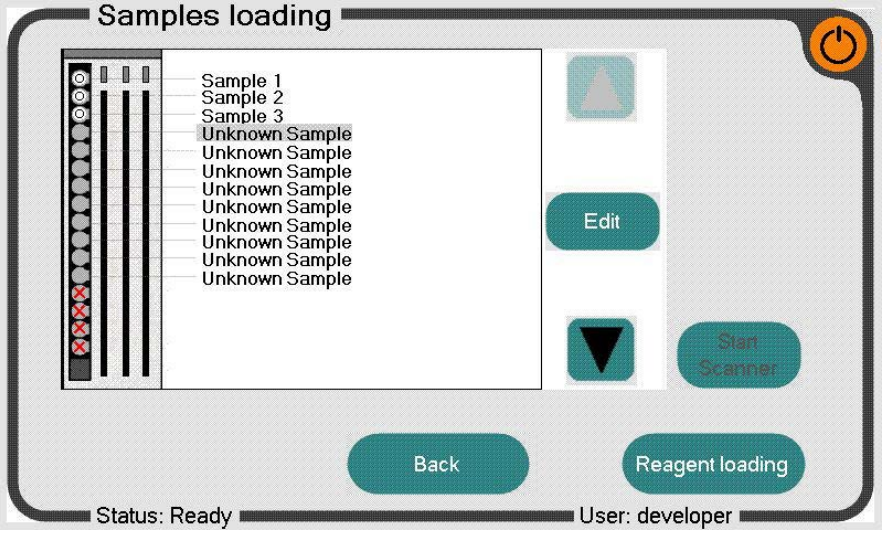


Figure 7: “Sample-loading” screen of the InviGenius® software

Please add the samples to the rack.

It should be directly used primary tubes provided from the blood collection, blood bank, etc.

If the samples are not provided in primary tubes, please prepare the sample rack with primary tubes that are prefilled with blood samples from which the genomic DNA shall be extracted. Sample tubes are not provided with the kit and can be ordered at e.g. Sarstedt (order nr.: 55.476, 5 ml tubes, 75x12 mm, PS) or see recommendation at page 9, chapter: reagents and equipment to be supplied by user.

For each reaction a sample volume of 200 µl is processed. It is necessary that the total provided sample volume should be at least 1000 µl to ensure stable processing. If the primary tubes are completely filled with blood please premix the tube to ensure a homogenous solution before inserting the tube into the sample rack.

Please decap the tubes before transfer to the loading rack.

Please take care, that only 12 positions of the sample rack can be processed per run due to the limited number of wells per row of the plastic ware. For correct identification of the sample tubes,

the sample bar code labels - must face to the bar code scanner window located at the right side of the loading bay.

After inserting the sample rack at the very left lane of the loading bay, an updated screen shows 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 use of the arrow fields, followed by the Edit button.

After a certain time the bar code scanner is inactivated. In that case the user has to restart the scanner with the “START SCANNER” button.

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

Reagent Loading:

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

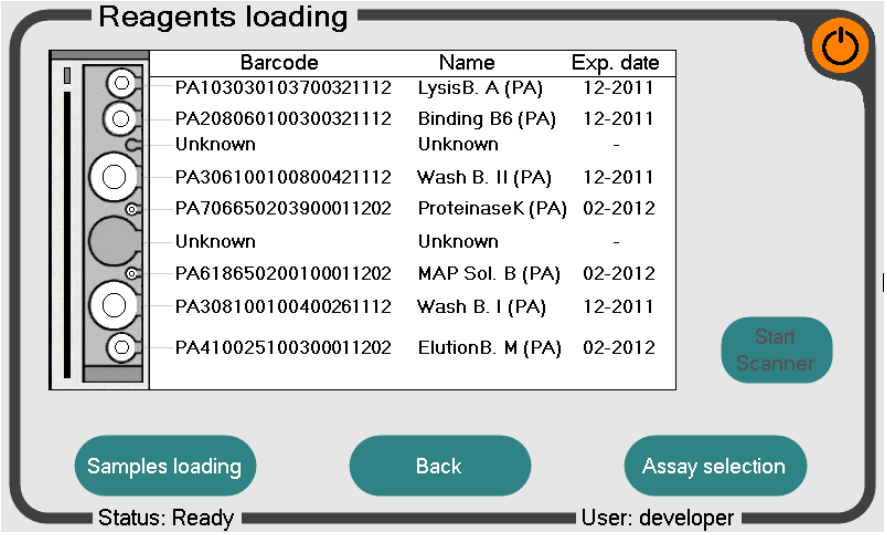


Figure 8: “Reagent-loading” screen of the InviGenius® software

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.

Add the lids of the bottles on a safe place.

The order of the inserted reagents is not crucial because the type and position of a reagent is identified by the unique bar code. However, the possible loading positions are limited by the size of the used bottles.

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

Assay Selection

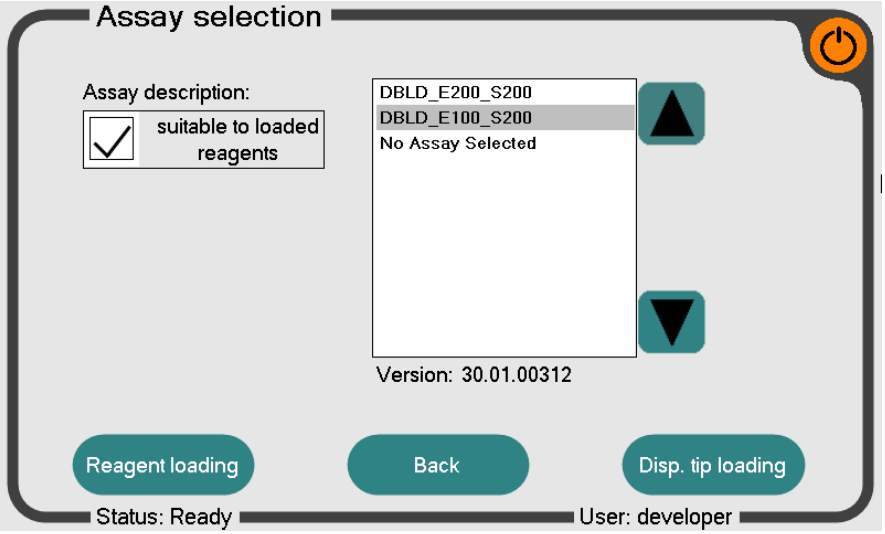


Figure 9: "Assay Selection" screen of the InviGenius® software

Select the appropriate protocol and proceed with disposable tip loading.

Disposable Tip Loading:

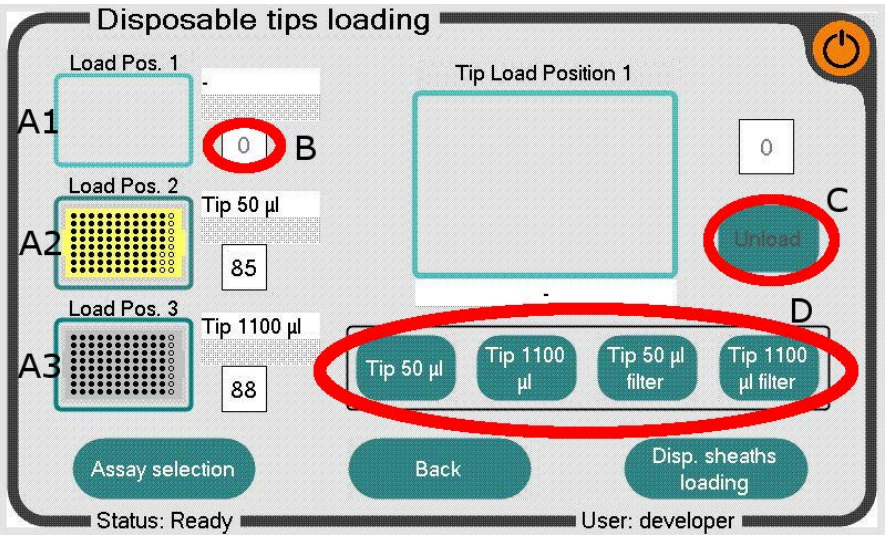


Figure 10: Disposable tip loading screen

There are three tip rack positions on the InviGenius® system (Fig. 10, A1-A3; corresponding to Fig. 3, D1-D3). 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 (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

Each position can be filled either with 50 µl or 1100 µl filter or non-filter tips. For the blood assay only 1100 µl filtered tips are needed.

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

All protocols should be used in combination with conductive filter tips to ensure efficient prevention of cross-contaminations of samples and reagents. STRATEC Molecular will take no guarantee or responsibility if contaminations occur due to the use of non-filter tips.

Note: ***Disposable tips are not supplied within the kit. We recommend only the use of validated conductive tips, which can be ordered at the STRATEC Molecular company. Be sure that you use conductive tips otherwise the liquid level detection will not work properly!***

For order information see page: 26

Disposable Sheaths Loading:

The sheaths are used as protection devices for the magnetic rods. The sheaths are picked up automatically during the run and provided in the kits.

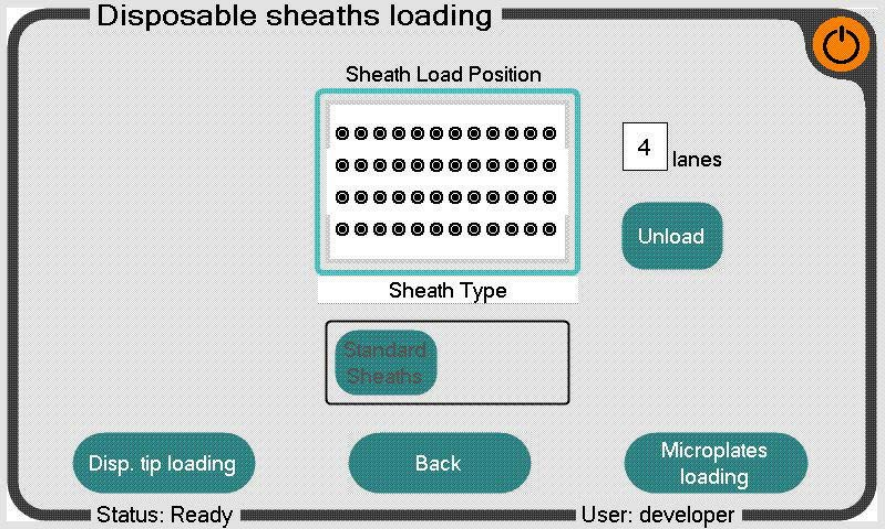


Figure 11: Disposable sheaths loading screen

The loading procedure of the disposable sheaths works analogous to the disposable tip loading screen. For a run, always 12 disposable sheaths (one row in the sheaths rack) are used, regardless of the sample number processed. This is done, to assure that all rods are protected against contaminations. In general, the number of sheaths supplied within the kit are sufficient for the amount of runs printed on the kit package. If you are lacking sheaths, they can be ordered separately at STRATEC Molecular.

For order information see page: 26

Comparable to the disposable tips loading the number of rows left in the tip rack you must be defined by pressing on the number area. Please ensure the disposable sheaths are loaded (and displayed) consistent to the manually loaded sheaths in the rack to ensure correct sheaths pick up. Don't remove single disposable sheaths within a row of the sheaths rack if less then 12 samples are processed within one run because there is a sheaths detection sensor installed in the device. If less than 12 sheaths picked up by the instrument an error will occur and all picked up sheath will be discarded into the waste before the next row of sheaths will be picked up for testing.

To avoid contaminations, we strongly recommend to not wash and reuse sheaths!

Plate Loading:

Analogous to the previous loading screens, the incubation, working and elution plate are loaded within the plate loading screen (Figure 12).

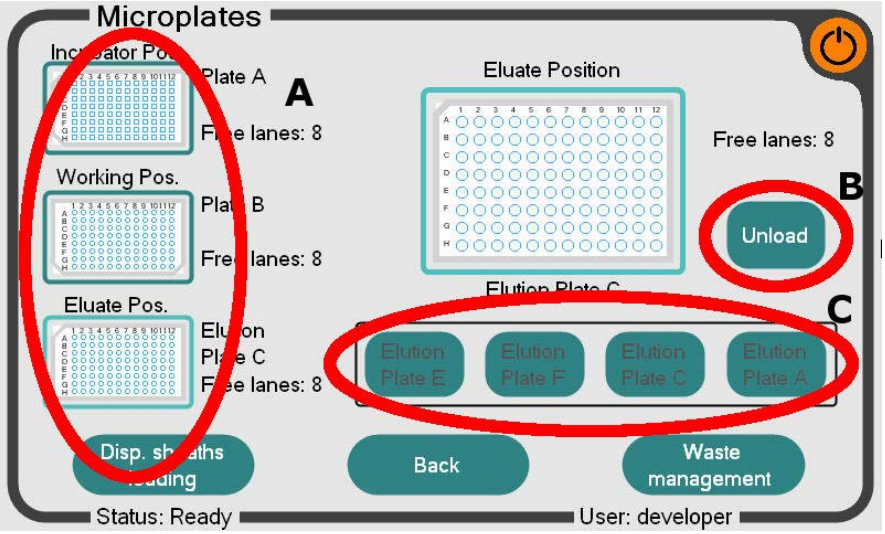


Figure 12, Plate loading screen

In general, Incubator Plates A and Working Plates A (provided) are used in the incubator and working position. In the eluate position a Elution Plate C is used.

Used plates can be unloaded and reloaded by:

1.) Pressing the plate position directly (A). The software will focus the plate position on the main screen.

2.) Pressing the “Unload” button (B)

3.) The plate can be reloaded by pressing on the offered plate in (C).

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

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

To avoid contaminations, we strongly recommend to not wash and reuse old plates!

Note: ***The blood assay uses 1100 µl filter tips only. There is no need to use any 50 µl filter tips using the blood assays.***

Waste management

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

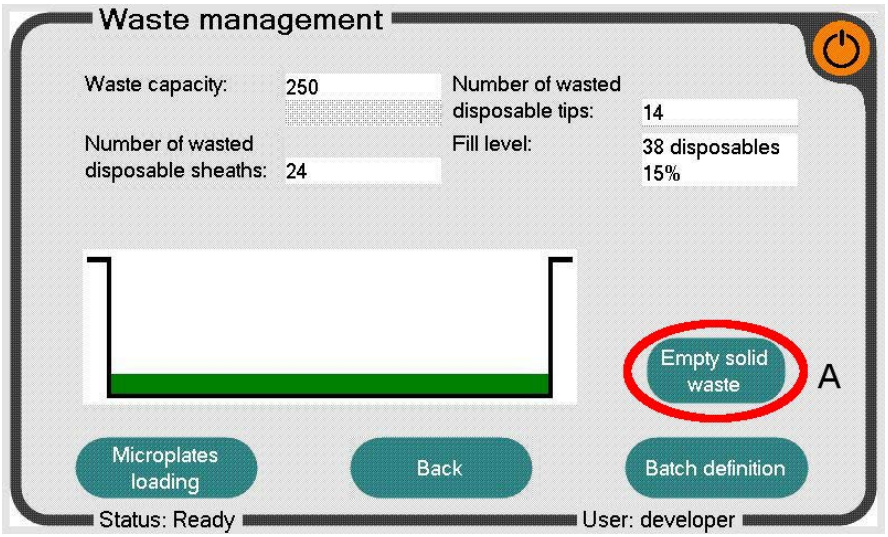


Figure 13: Waste-management-screen

If you have evacuated the waste tray, please use the “Empty solid waste” button (A).
Use only waste trays provided by STRATEC Molecular, (see ordering information, page 26)

Batch definition

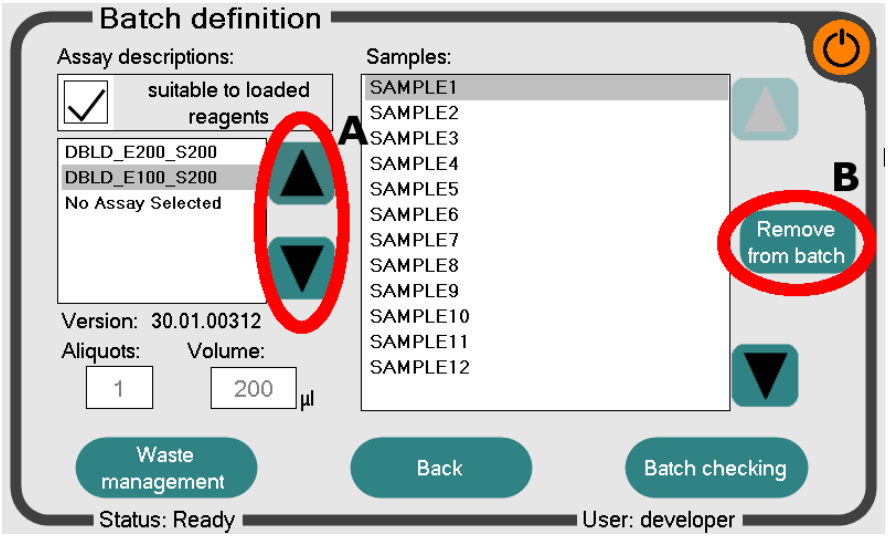


Figure 14: Batch-definition-screen

Please select the desired assay and check the samples you want to process in this run. You can select the assays by using the two arrow buttons (A). By default, all loaded samples are selected. If you want to exclude samples from the batch you can exclude them by selecting and clicking on the “Remove from batch” button (B).

Batch checking

This screen shows a summary of all checked disposables, samples and buffers in one screen. Please make sure that everything is loaded correctly. In case of any errors, the problem is highlighted in red fonts. If no errors during the loading steps occurred, proceed by pressing the button “Batch processing”.

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

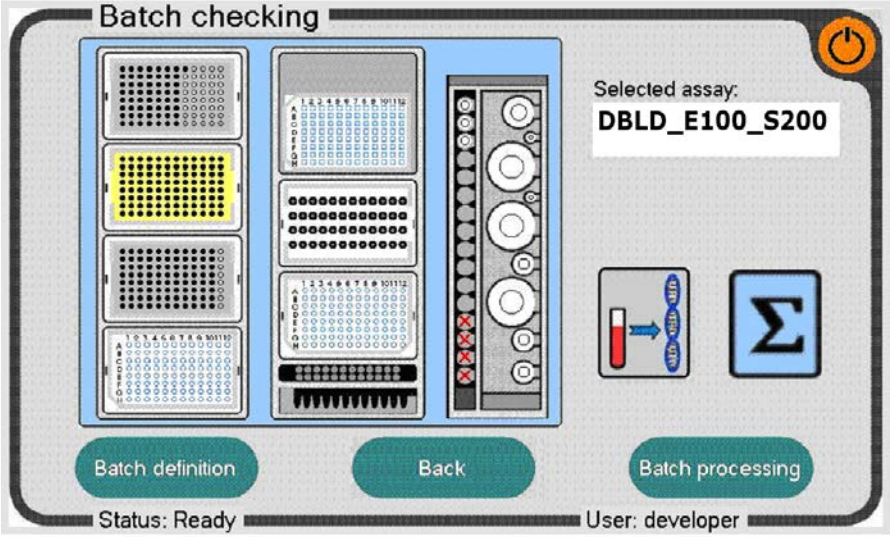


Figure 15: Batch-definition-screen

Batch processing

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

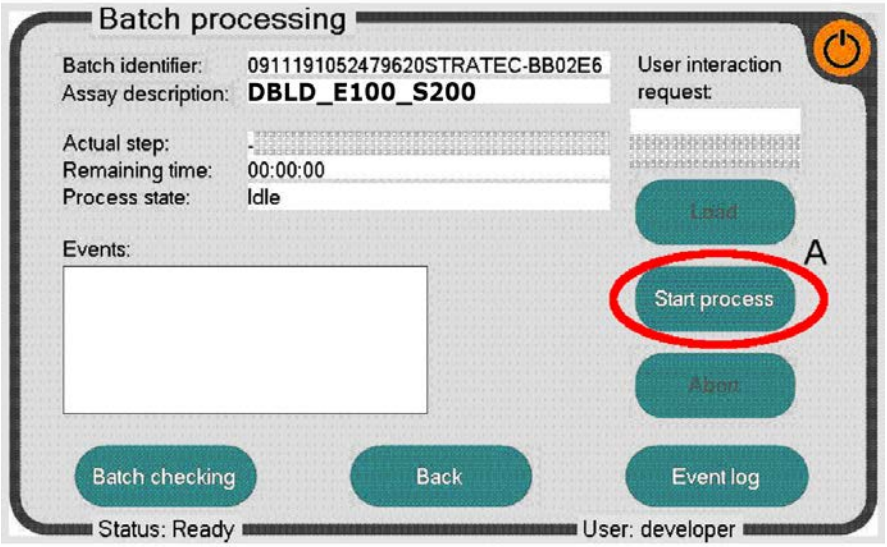


Figure 16: Batch-processing-screen

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

Note: ***The complete process will take approximately 60 minutes.***

After a run

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

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

Daily maintenance (UV decontamination)

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

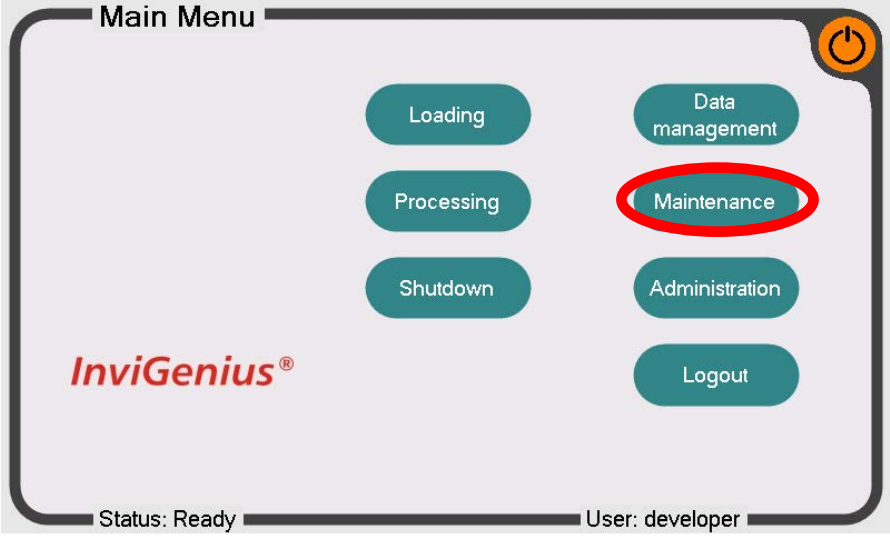


Figure 17: Main screen of the InviGenius® software

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

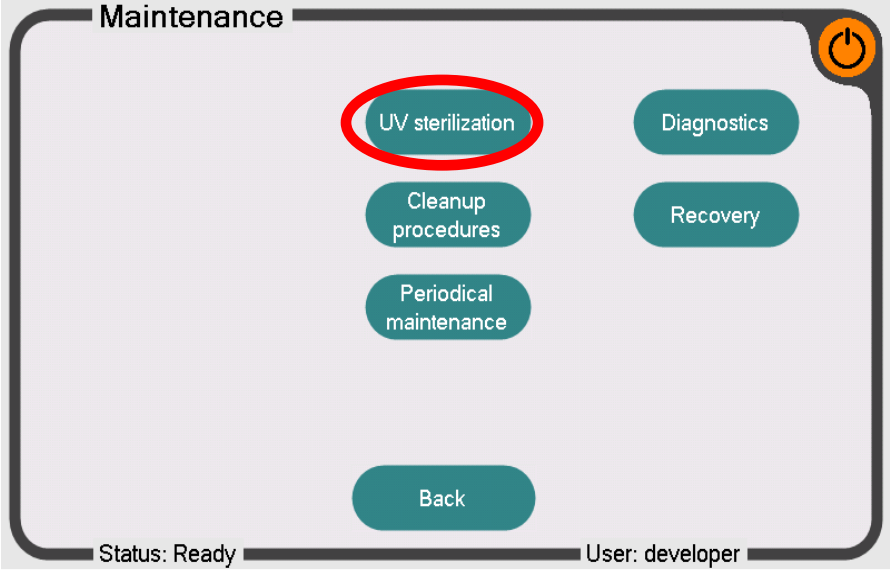


Figure 18: Maintenance screen of the InviGenius® software

In the UV sterilization menu adjust the sterilization time (A) and finally press the “Start” button (B). Make sure that the front door is closed during the decontamination time to prevent UV radiation released in the lab.

Warning: ***UV radiation is harmful. It causes serious burns of the skin and leads to irreparable damage of the eyes and skin. Ensure that no lab employees is submitted to direct UV light. Keep the instrument door always closed during the decontamination process.***

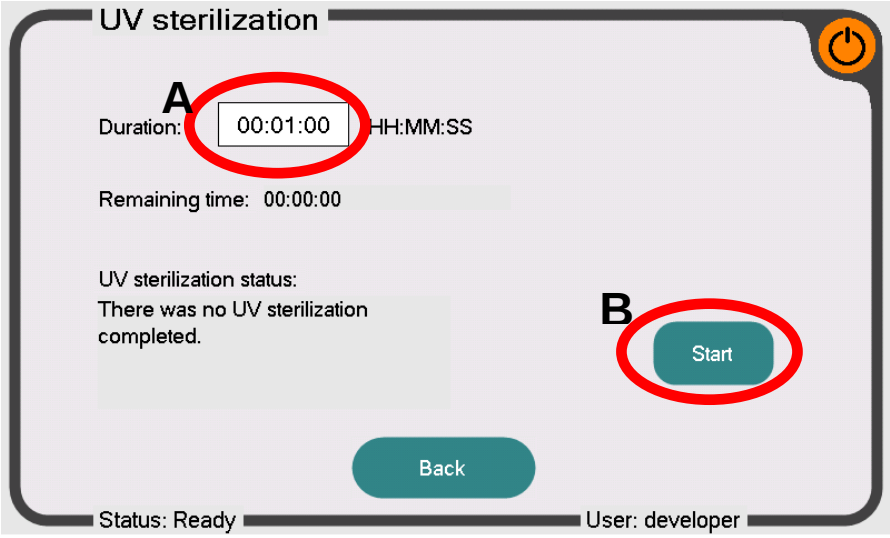


Figure 19: UV sterilization screen

When the sterilization 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 to decontaminate the device daily.

Appendix

Example data

Genomic DNA was isolated using the InviMag® Blood DNA Mini Kit/ IG from 200 µl of whole human blood, stored at – 20°C. The isolated DNA was analyzed by agarose gel electrophoresis and the DNA was suitable for real-time PCR amplification which is demonstrated by the successful amplification of the GAPDH-fragment in the samples.

figure A

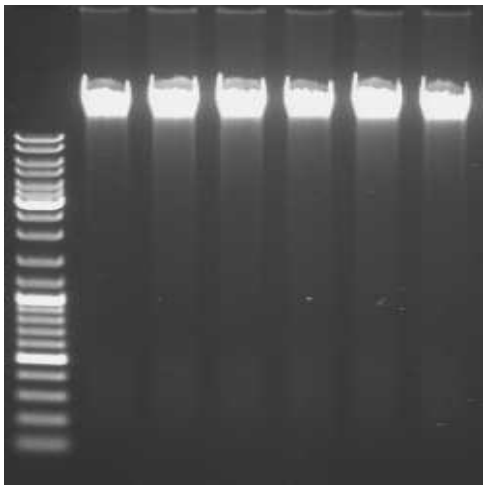
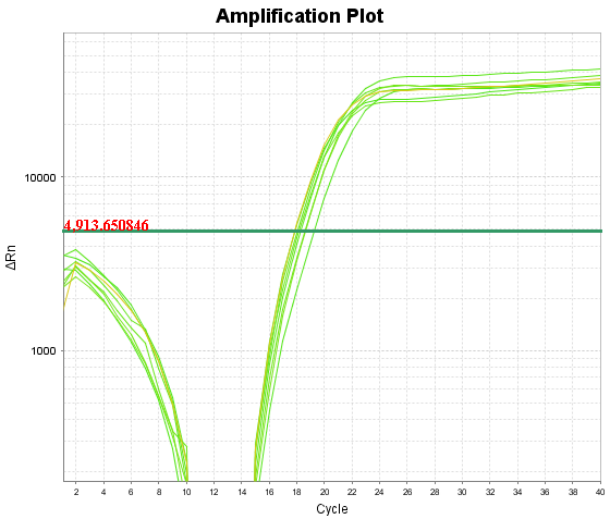


figure B



The average purity of all isolated samples is about 1.7± 0.1 and represents pure DNA. The overall yield of all samples derived from 200 µl blood is about 6 ± 0.4 µg. The elution volume was 200 µl.The measurement was performed with the Nanodrop 1000 instrument. 2 µl eluate was used for measurement.

The performed PCR proves that no inhibition of any blood sample was observable. All samples were fully functional and showed a consistent picture. 2 µl eluate were used for a PCR reaction. The PCR was done on a StepOnePlus cycler from Applied Biosystems.

The CT values derived from the PCR illustrated in Fig.B. All CT values belonging to one sample type are in the same range with a very low standard deviation. This result demonstrates the excellent conformity of the samples processed by the InviGenius system.

General notes on handling DNA

Nature of DNA
The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure its functionality in various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long-template PCR, and construction of cosmid libraries.

Handling fresh and stored material before the extraction of DNA
For the isolation of genomic DNA use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at -70°C. This procedure minimizes degradation of crude DNA by limiting the activity of endogenous nucleases.

Storage of DNA
Store genomic DNA at 2-8°C. Storing genomic DNA at --20°C can cause shearing of DNA, particularly if the DNA is exposed to repeated freeze-thaw cycles. Plasmid DNA and other small circular DNAs can be stored at 2.8°C or at -20°C.

Troubleshooting

Problem	Probable cause	Comments and suggestions
pipetting distribution errors	pipetting of **Proteinase K** failed	make sure that you have resuspended the lyophilized **Proteinase K** with the appropriate volume of water before use
	samples transfer failed / incomplete	the sample tube must contain at least 1000 µl sample
	reagent / buffer transfer failed / incomplete	ensure that the supplied **Wash Buffers** are filled up with ethanol properly do not reuse bottles more often than described in Tab.1
low concentration of extracted DNA	blood components settled	in case of large sample volumes (>>2 ml) carefully premix the sample tube before inserting it into the sample rack
	no/ too much ethanol added to **Wash Buffers**	ensure that the **Wash Buffer** have been filled up with ethanol properly as indicated in Tab. 1
degraded or sheared DNA	incorrect storage of starting material	ensure that the storage of starting material was correctly avoid multiple freezing and thawing cycles of the material
	old material	ensure that the starting material is fresh or stored under appropriate condition (for long time storage at –20°C)! avoid multiple thawing and freezing cycles of the material old material often contains degraded DNA
no assay selectable	combination of reagents from different kits	please make sure that only reagents belonging to one kit type are used. a combination of reagents belonging to different kit types will not work
eluted DNA is brown colored	small part of the magnetic particles are left in the elution	centrifuge the eluate plate at full speed for 1 min and transfer supernatant to a new plate don't use the 100 µl eluate assay protocol for "normal" blood samples. This protocol is designed for blood samples with a very low leukocyte content

Order information

Product	Package size	Order-Nr.
InviMag® Blood DNA Mini Kit/ IG	8 x 12 preps	2431120100
Related products		
InviMag® Blood DNA Midi Kit/ IG	8 x 12 preps	2431720100
Invisorb® Spin Blood Mini Kit	50 preparations	1031100200
Invisorb® Spin Blood mini Kit	250 preparations	1031100300
Invisorb® Spin Blood Midi Kit	50 preparations	1031110300
Invisorb® Spin Blood Midi Kit	250 preparations	1031110500
InviGenius® and consumables		
InviGenius®	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