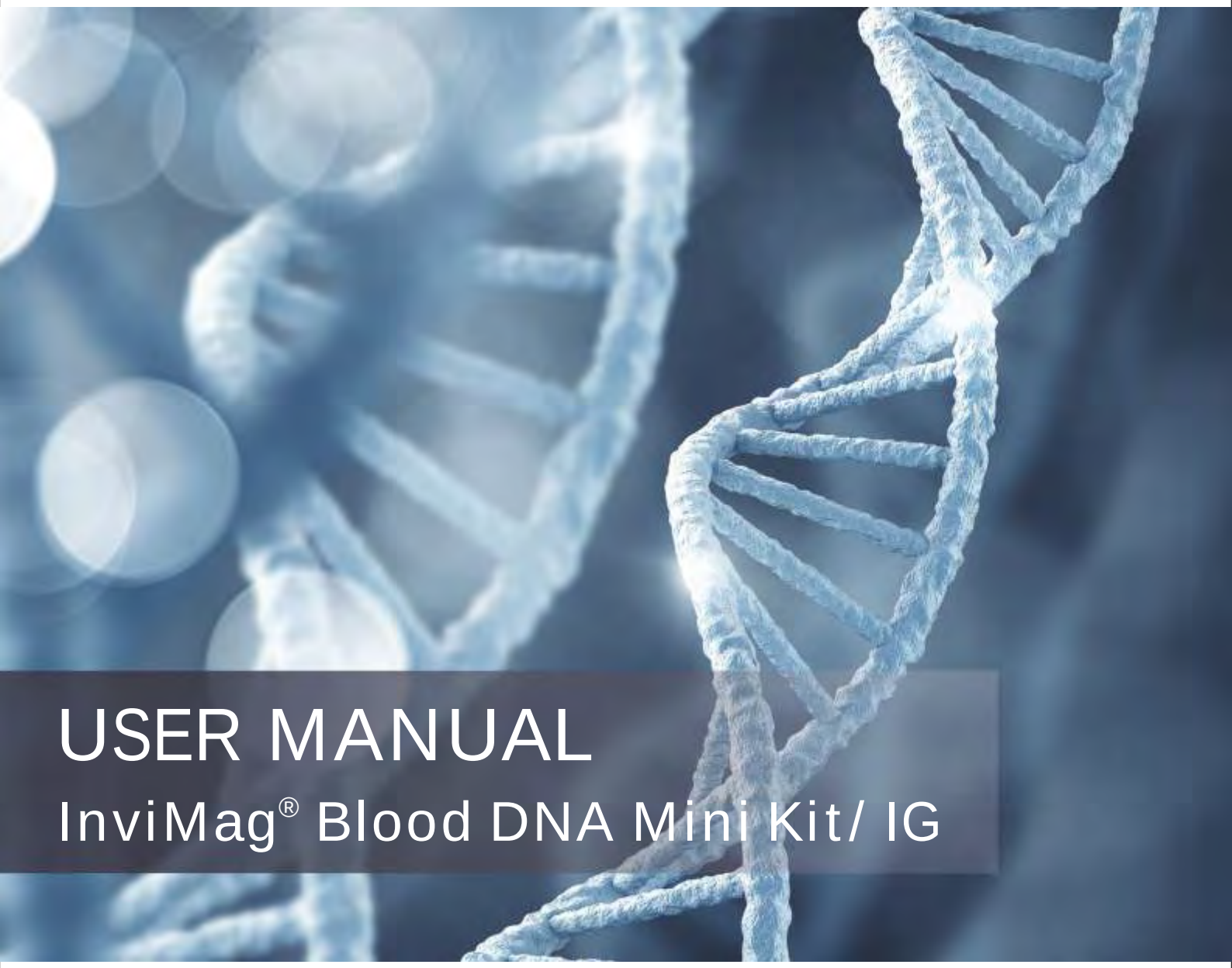
INVITEK
Molecular



USER MANUAL

InviMag® Blood DNA Mini Kit/ IG

for automated purification of DNA from 200 µl whole blood samples
with magnetic beads

CE IVD

REF 2431120100

Invitek Molecular GmbH · D-13125 Berlin

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 (genomic and mitochondrial) DNA from 200 µl whole blood samples (EDTA or citrate stabilized *but not heparin*). The kit is designed for 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, uniform size distribution, good suspension stability and are highly suitable for high-throughput processing.

Due to the high purity, the isolated DNA is ready-to-use in a broad panel of 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 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 cultured or isolated cells, from tissue, blood cards, dried bloodstains or 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.

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® Blood DNA Mini Kit /IG

Component	8 x 12 preps	Reagent sufficient for
Catalogue No.	2431120100	
Lysis Buffer HLT	2 x 30 ml	per bottle: 48 samples (in max. 6 runs)
Proteinase S	3 ml	96 samples (in max. 12 runs)
MAP Solution B	2 x 2.6 ml	per tube: 48 samples (in max. 6 runs)
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 80 ml)	96 samples (in max 12 runs)
Ethanol (fill with 96 - 100% Ethanol)	empty bottle (final volume 120 ml)	96 samples (in max 12 runs)
Wash Buffer II	40 ml	96 samples (in max 12 runs)
Elution Buffer	100 ml	96 samples (in max 12 runs)
Incubation Plate A	1	8 runs per plate
Working Plate A	4	2 runs per plate
Elution Plate E	1	8 runs per plate
Sheath Box	1 (2 racks á 48 sheaths)	4 runs per rack
Microtube Cap	8	
Sealing Foils	4	
Incubator Stripe Foils	1	
Initial steps	Add 80 ml of 99.7% Isopropanol to the bottle **Binding Solution** Add 120 ml of 96 - 100% Ethanol to the bottle **Ethanol**	

Symbols

 Manufacturer

 Lot number

 Catalogue number

 Expiry date

 Consult operating instructions

 Temperature limitation

 Do not reuse

 Humidity limitation

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

Storage

All buffers and kit contents of the **InviMag® Blood Mini Kit/ IG** 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). All bottles and tubes should be appropriately sealed after the run and stored at room temperature.

Quality control and product warranty

Invitek Molecular warrants the correct function of the **InviMag® Blood Mini Kit/ IG** for applications as described in this manual. Purchasers 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 lot connected problem is investigated Invitek Molecular will replace the product 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® Blood Mini Kit/ IG** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

In case of any question or problem regarding any aspect of **InviMag® Blood Mini 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 www.invitek-molecular.com.

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 DNA Mini Kit /IG** is designed for fully automated extraction and purification of genomic DNA from up to 200 µl whole blood ranging from 1–12 samples per run 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 samples. For reproducible and high yields, the appropriate sample storage is critical (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, which have to be provided from user, are listed on page 10.

The procedure of the **InviMag® Blood DNA Mini Kit/ IG** has been optimized for the isolation of genomic DNA from up to 200 µl starting material. However, we advise to provide a sample volume of at least 600 µl per a 12 mm tube diameter (more if bigger tubes are used) 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 is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications 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 use of whole blood samples only. The isolation of DNA from other sources like stool, tissue, bacteria, fungi or virus was neither tested nor validated.

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

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

Lysis Buffer HLT

Warning

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

Proteinase S

Danger

H317-H318-P280-P305-P351-P338

H302: Harmful if swallowed.
H315: Causes skin irritation.
H317: May cause an allergic skin reaction.
H318: Causes serious eye damage.
H319: Causes serious eye irritation
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

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

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

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

The **InviMag® Blood DNA 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
50-200 µl fresh or frozen whole blood	2 - 10 µg, depends on the blood sample (storage and source)	about 90 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 at 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 contaminations and reagent cross-overs are 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 such as lysis, binding, washing and 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 process on **InviGenius®** is total in-process control – including e.g. completely reagent tracking „on the bottle“, shelf live, amount whereas a detailed inventory checks helps to eliminate human errors, data storage, backup and archiving. Due to the high purity, the eluted total (genomic and mitochondrial) DNA is ready to use for a broad panel of downstream applications:

- PCR, qPCR
- Sequencing
- Restriction Enzyme Digestion
- HLA Typing
- Southern Blot

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.

Sampling and storage of starting material

For reproducible and high yields, 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 whole 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 2-8°C. For long-term storage, we recommend freezing the samples at -20°C or -80°C. Avoid multiple thawing and freezing cycles of the sample(s) before isolating the DNA. Various different blood collection tubes (e.g. Sarstedt, Greiner) and anticoagulants (except heparin) can be used to collect blood samples for the **InviMag® Blood DNA Mini Kit /IG** procedure

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® 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 of the bead bound DNA and elimination of ethanol
- elution of genomic DNA

After lysis, the DNA binds to the magnetic beads whereas contaminations and enzyme inhibitors are efficiently removed during the following three washing steps. Finally, highly purified DNA is eluted in Elution Buffer.

Lysis
Samples are lysed at chaotropic conditions in the Incubation Plate A at elevated temperatures in the presence of **Lysis Buffer HLT** and **Proteinase S**.

Binding of the genomic DNA
The DNA is bound efficiently to the surface of the **MAP Solution B** (paramagnetic beads) after addition of **Binding Solution**.

Removing residual contaminants
Contaminants are efficiently removed in the Working Plate A using Wash Buffers while the DNA remains bound to the magnetic beads.

Elution
The DNA is finally eluted in **Elution Buffer**. The eluted DNA is ready-to-use in different subsequent downstream applications like PCR amplification, restriction enzymes analysis, southern hybridizations, HLA typing, etc.

Yield and quality of genomic DNA

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

Typically, a volume of 200 µl of a whole blood sample from a healthy individual without 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 in the range of 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 slightly reduced.

Important notes

Important points before starting a protocol

Immediately upon receipt, inspect the product and its components as well as the package for any apparent damages and correct quantities. If there are any unconformities 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, because their use may lead to poor kit performance.

- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard contaminated gloves immediately
- Never combine components of different kits
- Avoid microbial contamination of the kit reagents
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are prepared
- This kit should only be used by trained personnel

Preparing reagents and buffers

Before starting a run, equilibrate all reagents at 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, Wash Buffer II and Elution Buffer are ready-to-use. The empty bottles have to be filled like shown in the following table.

8 x 12 DNA-extractions:
Add 80 ml of 99.7% Isopropanol to the bottle **Binding Solution.**
Add 120 ml of 96 - 100% Ethanol to the bottle **Ethanol.**

Reagents and equipment to be supplied by the user

- Measuring cylinder (250 ml)
- Conductive pipette tips (see ordering information, page 26)
- Disposable gloves
- 1 x PBS
- ddH_2O
- Vortexer
- 96-100% Ethanol
- Primary tubes (e.g. see below)
- > 99,7% Isopropanol*

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

*** Possible suppliers for Isopropanol**

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

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

Possible primary tubes, manufacturer, Cat. No.

Venosafe, 5.5 ml, Ref, VF-076SDK, Terumo
Vacuette, 2 ml, Ref, A110500l, Greiner bio-one
Vacuette, 9 ml, Ref, 455036, Greiner bio-one
BD Vacutainer, 2.7 ml, Ref, 363048
BD Vacutainer, 6 ml, Ref, 367864
BD Vacutainer, 10 ml, Ref, 367525
BD Vacutainer 5.0 ml, Re
Sarstedt Monovette, 8.5 ml
PS Tube Sarstedt 5 ml, Ref: 55.476
Sarstedt Monovette 4.5 ml
Sarstedt Monovette 7.5 ml
Sarstedt Monovette 9.0 ml

Important indications

1. Minimum volume of samples in primary tubes

The procedure of the **InviMag® Blood DNA Mini Kit /IG** has been optimized for the isolation of genomic DNA from up to 200 µl whole blood.

For the assays with **automated transfer** (DBLD_E100S200_**AT** and DBLD_E200S200_**AT**) we advise to provide at least 550 µl blood per sample tube to prevent pipetting and distribution errors during processing.

2. Sample volume smaller than 200 µl

For samples smaller than 200 µl please adjust the volume up to 200 µl using 1x PBS for the assays with **manual transfer** (DBLD_E100S200_**MT** and DBLD_E200S200_**MT**)

3. Elution volume

Assays using different elution volumes are provided with this kit.

Elution volume 100 µl: DBLD_**E100**S200_MT and DBLD_**E100**S200_AT

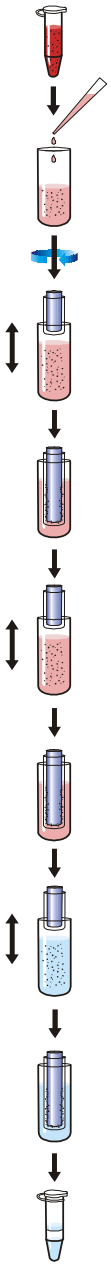
Elution volume 200 µl: DBLD_**E200**S200_MT and DBLD_**E200**S200_AT

Typically, 200 µl eluate yields in app. 2-10 µg DNA.

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.

Scheme of the InviMag® Blood DNA Mini Kit/ IG

	200 µl sample will be mixed with 200 µl **Lysis Buffer HLT** and 20 µl **Proteinase S.** Incubation at 56°C for 10 min is performed 260 µl **Binding Solution** *(follow preparing instructions)* and 40 µl **MAP Solution B** are added to the lysate DNA binds to magnetic particles magnetic separation washing of the particle fixed genomic DNA in different Wash Buffers and steps magnetic separation elution of genomic DNA in 100 µl / 200 µl **Elution Buffer** magnetic separation (removal of beads) transfer of eluate to the eluate plate

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).*
*For assays with automated sample transfer: To prevent possible distribution errors it is highly recommend to provide at least **550 µl** of sample in the sample tube to ensure stable processing.*

Attention: *Make sure that the Binding Solution and the Ethanol bottle are prepared following the instructions* on *page 9.*

Extraction of genomic DNA from whole blood using the different provided Assays

For **automated sample transfer (AT)** load the Sample Rack with sample tubes, sample volume minimum **550 µl**

For manual sample transfer (MT) load **200 µl Blood** directly into the first free lane of Incubator Plate A starting with A1 and ending with A12.

Place the Plate into the Incubator. If sample tubes are barcoded place the tubes in the sample rack in the same sequence they are located in Incubator Plate A.

	Sample transfer	**Sample volume**	**add**	**Eluate Volume**
DBLD_E100S200_AT	**automated**	bigger or equal 550 µl	-	100 µl
DBLD_E200S200_AT	**automated**	bigger or equal 550 µl	-	200 µl
DBLD_E100S200_AT	**automated**	smaller than 550 µl	Bring sample to 550 µl with 1x PBS.	100 µl
DBLD_E200S200_AT	**automated**	smaller than 550 µl	Bring sample to 550 µl with 1x PBS.	200 µl
DBLD_E100S200_MT	**manual**	smaller than 200 µl	Manually transfer 200 µl sample into Incubation Plate D beginning with position A1	100 µl
DBLD_E200S200_MT	**manual**	smaller than 200 µl	Manually transfer 200 µl sample into Incubation Plate D beginning with position A1	200 µl
DBLD_E100S200_MT	**manual**	smaller than 200 µl	Bring sample to 200 µl with 1x PBS. Manually transfer 200 µl sample into Incubation Plate D beginning with position A1	100 µl
DBLD_E200S200_MT	**manual**	smaller than 200 µl	Bring sample to 200 µl with 1x PBS. Manually transfer 200 µl sample into Incubation Plate D beginning with position A1	200 µl

Important: *DBLD_E100S200 assay should only be used if whole blood with a very low content of leukocytes will be used*

General overview of the InviGenius® PLUS

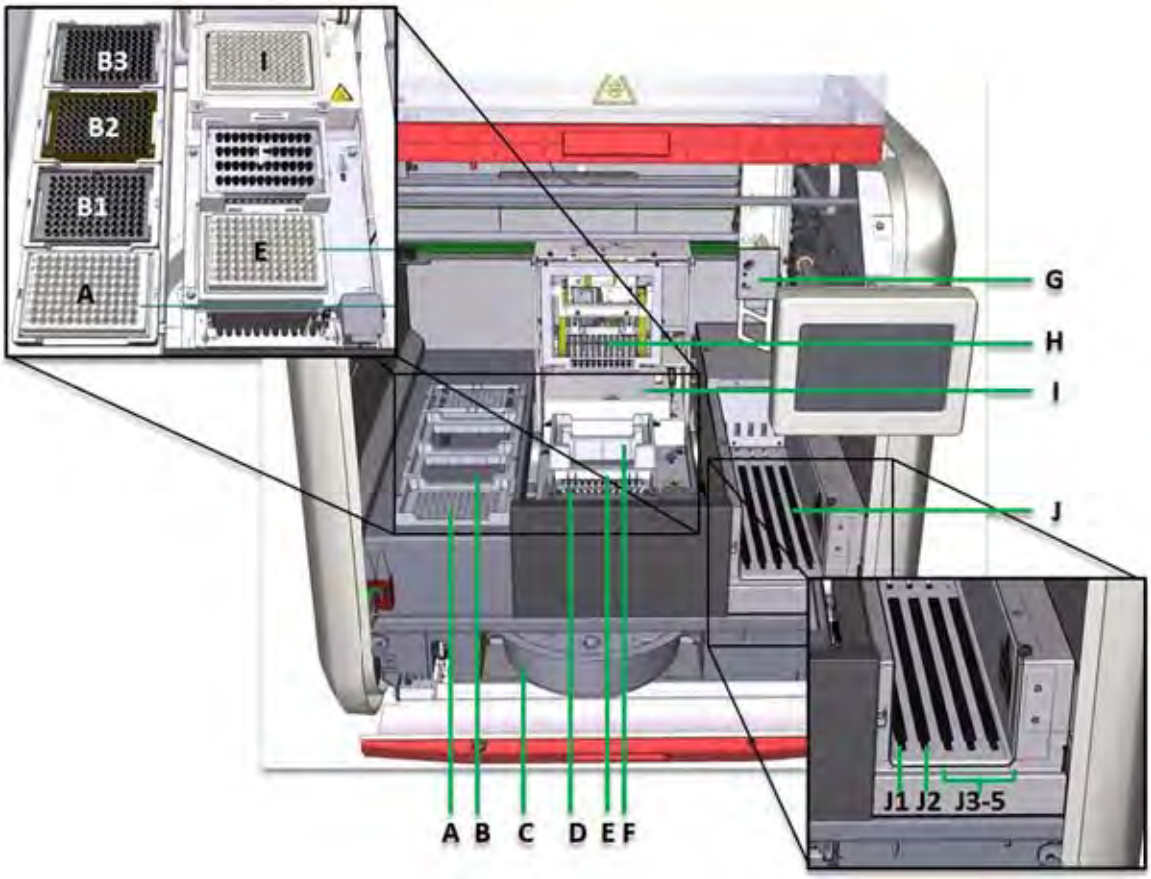


Figure 1: Frontal view of the InviGenius® PLUS system

Fehler! Verweisquelle konnte nicht gefunden werden. 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® system

Preparing the reagents:

If a new kit is used, add ethanol to **Ethanol** (bottle) and isopropanol to the **Binding Solution** (bottle).

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 2). Login: use the provided user name and password.

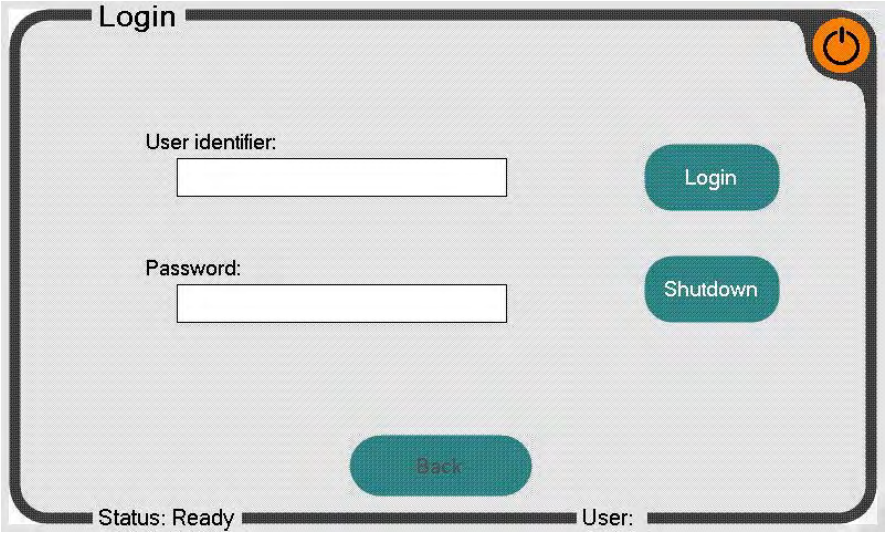


Figure 2: Login screen of the InviGenius® software

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

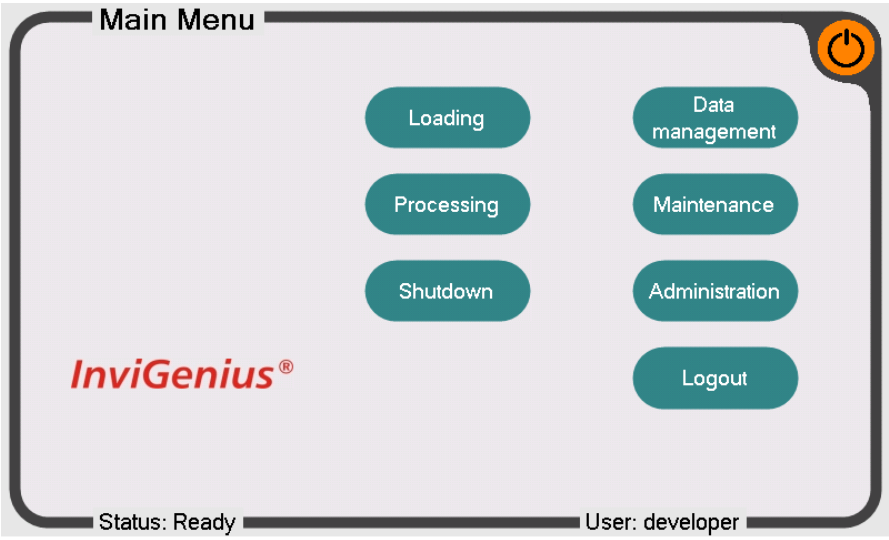


Figure 3: Main menu of the InviGenius® software

Sample Loading:

After selecting “Loading” the main loading screen appears.

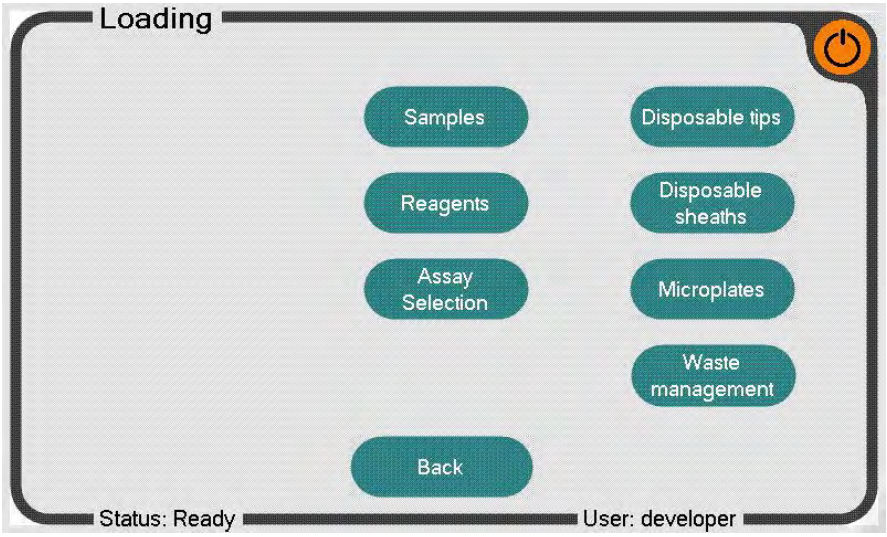


Figure 4: Loading screen of the InviGenius® software

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

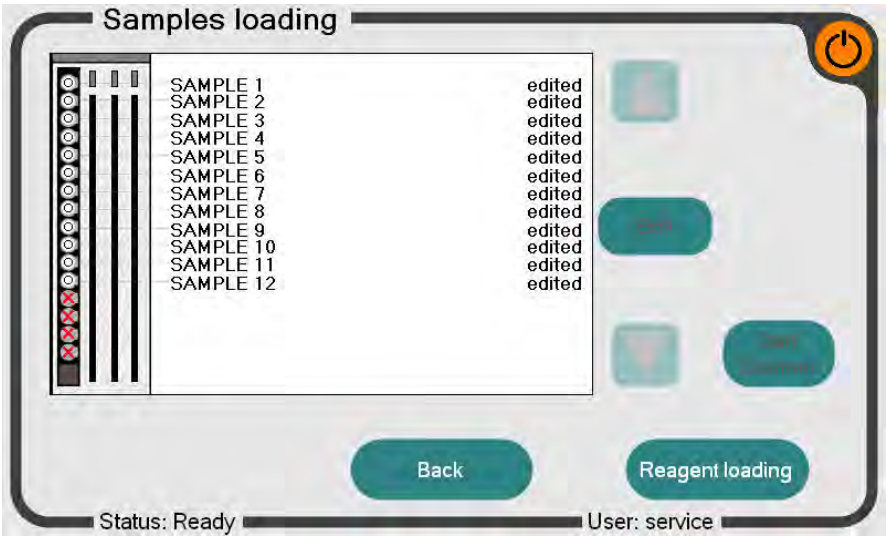


Figure 5: “Sample-loading” screen of the InviGenius® software

Automated Sample Transfer (AT)

Decap the sample tubes and insert into the sample rack. If possible, the primary tubes should be used directly as sample tubes. If the samples are not provided in primary tubes, please prepare the sample rack with primary tubes that are prefilled with samples from which the DNA shall be extracted. Sample tubes are not provided with the kit but can be ordered at e.g. Sarstedt (order no. 55.476, 5 ml tubes, 75x12 mm, PS) or see recommendation at page 10, chapter “Reagents and equipment to be supplied by user”.

For each reaction, a sample volume of 200 µl is processed. However, it is recommended that the provided sample volume should be at least **550 µl** to ensure stable processing. Be aware that only the first 12 positions of the sample rack can be processed due to the limited number of wells per row of the plastic. For correct identification of the sample tubes the unique bar codes must face to the bar code scanner located at the right side of the loading bay.

After inserting the sample rack in the very left lane of the loading bay, an updated screen will show the identifiers read from the sample bar codes (Figure 5). In case of unsuccessful sample identification, remove the rack, check the bar code orientation, and reinsert the rack slowly. It is also possible to rename the samples by selecting the corresponding sample by using the arrow fields, followed by the “Edit” button.

Manual Sample Transfer (MT)

For manual sample transfer pipet **200 µl Blood** directly into the first **free** lane of Incubator Plate A starting with A1 and ending with A12. If row A of the Incubator Plate A has already been used in a previous run load the sample starting with B1 and ending with B12, etc.

Place the Plate into the Incubator. If sample tubes are barcoded place the tubes in the sample rack in the same sequence they are located in Incubator Plate A. After inserting the sample rack in the very left lane of the loading bay, an updated screen will show the identifiers read from the sample bar codes (Figure 5). In case of unsuccessful sample identification, remove the rack, check the bar code orientation, and reinsert the rack slowly. It is also possible to rename the samples by selecting the corresponding sample by using the arrow fields, followed by the “Edit” button.

After a certain time (about 5 min) the bar code scanner is inactivated. In that case the user has to restart the scanner with the “START SCANNER” button if the loading procedure is not finished.

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

Reagent Loading:

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

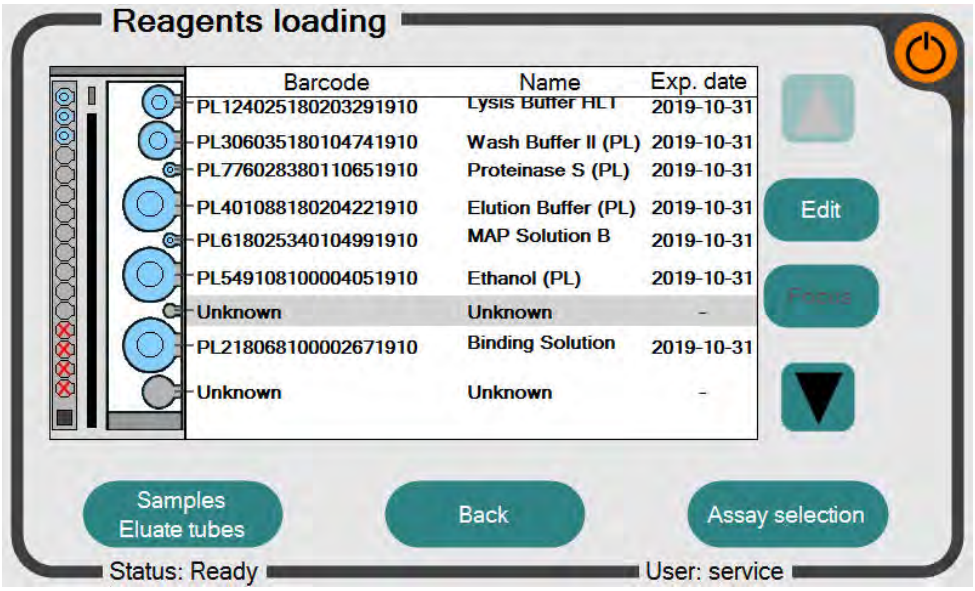


Figure 6: “Reagent-loading” screen of the InviGenius® software

Insert all provided reagents into the provided reagent rack of the InviGenius® system. Verify that the bar code labels face to the right side of the loading bay and decap the bottles and tubes. The order of the inserted reagents is not crucial because the type and position of a reagent is identified by the unique bar code. However, the possible loading positions are limited by the size of the 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

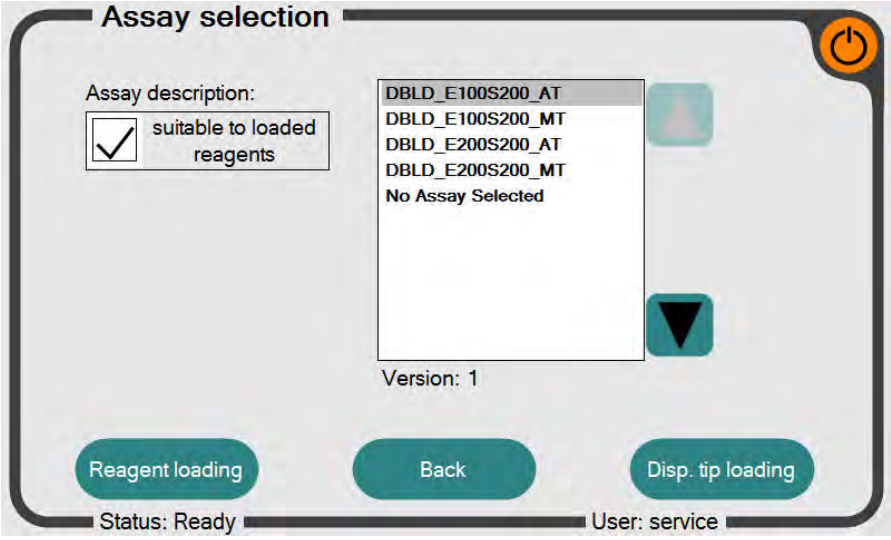


Figure 7: "Assay Selection" screen of the InviGenius® software

Select the appropriate protocol and proceed with disposable tip loading. If no assay file is visible one or more reagents were not recognized correctly during the reagent loading procedure go back to Reagent Loading and repeat the loading process.

Disposable Tip Loading:

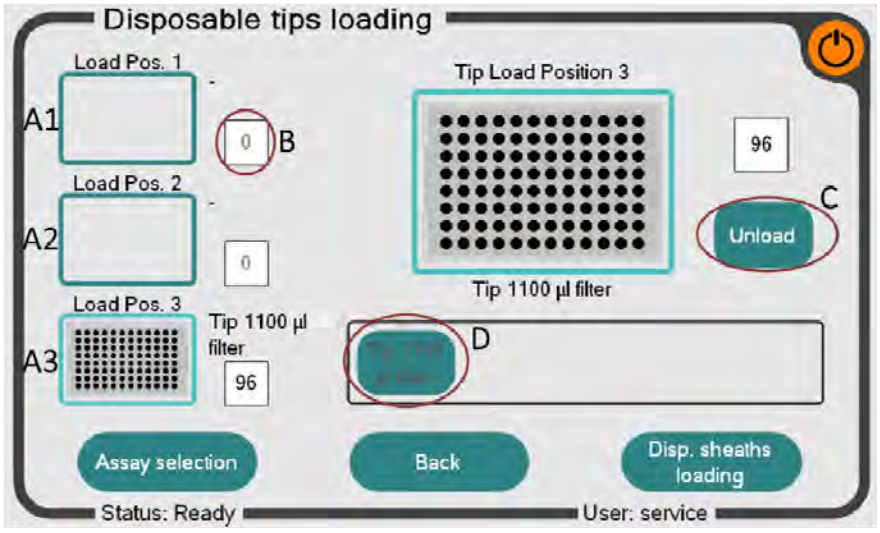


Figure 8: Disposable tip loading screen

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

Empty tip-racks can be unloaded and reloaded by:

1.) Pressing the Loading-Position directly (The software will focus 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

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 will irreparably 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 tips, which can be ordered at Invitek Molecular. Invitek Molecular offers 50 µl conductive tips (10x 96 pieces, order no. 5011120100) and 1100 µl conductive tips (10x 96 pieces, order no. 5011120200). Be sure that conductive tips are used otherwise the tip detection unit, installed in the pipetting unit, will reject the tips and no run will be possible.***

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.

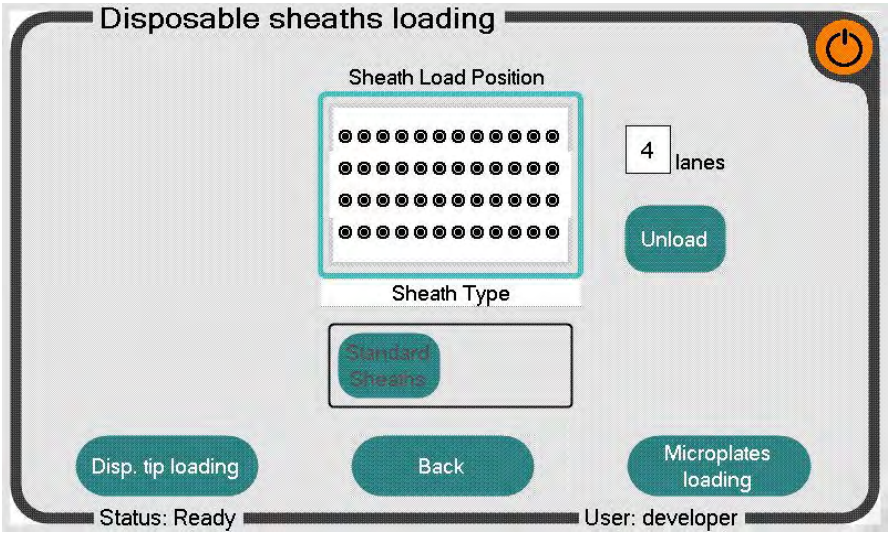


Figure 9: Disposable sheaths loading screen

The loading procedure of the disposable sheaths works analogous to the disposable tip loading screen. For a run, always 12 disposable sheaths (one row in the sheaths rack) are used, regardless of the processed sample numbers, 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, they can be ordered separately at Invitek Molecular (100 pieces bulk, order no. 5011120300 or 10 x 48 pieces, order no. 5011120400).

Comparable to the disposable tips loading it is possible to define the number of rows left in the tip rack by pressing on the displayed number area. Make sure that the disposable sheaths are loaded (and displayed) consistent to the manually loaded sheaths in the rack to ensure correct sheaths pick up. Do not remove single disposable sheaths within a row of the sheaths rack if less than 12 samples are processed within one run because there is a sheaths detection sensor installed in the device. If less than 12 sheaths picked up by the instrument a warning will be displayed and all picked up sheath 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/re-using any disposed sheaths!

Plate Loading:

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

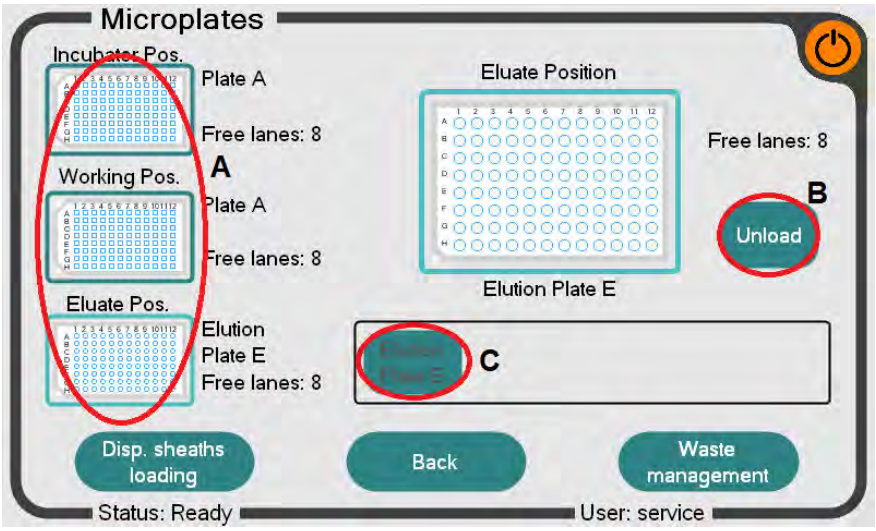


Figure 10, Plate loading screen

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

Used plates can be unloaded and reloaded by:

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

2.) Pressing the “Unload” button (B)

3.) The plate can be reloaded by pressing 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 not washing/re-using any disposed plates!

Waste management

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

Note: The waste is potentially infectious.

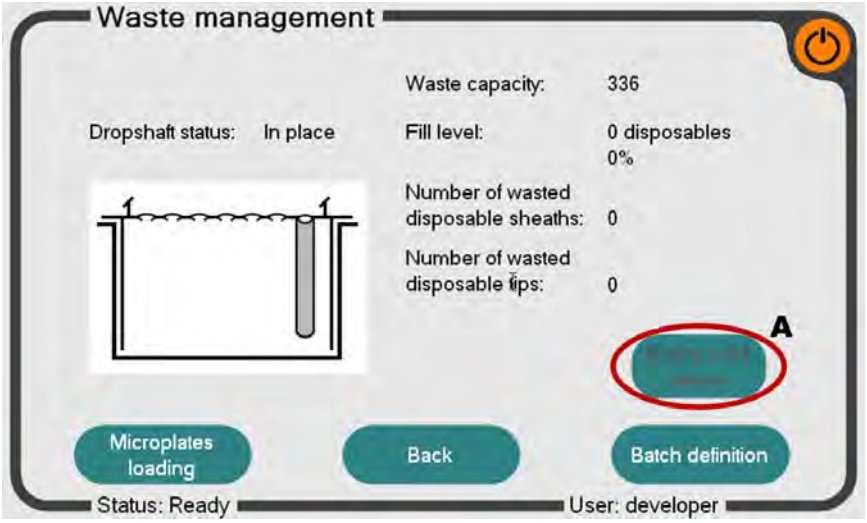


Figure 11: Waste-management-screen

If the waste tray (must be ordered separately from Invitek Molecular GmbH) was emptied / exchanged, please use the “Empty solid waste” button (A).

Batch definition

Please select the appropriate 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.

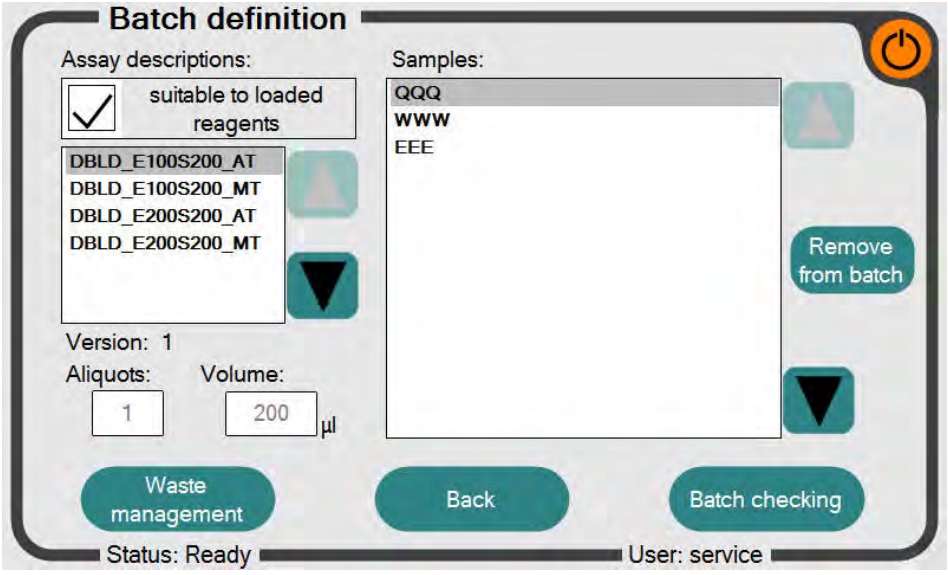


Figure 12: Batch-definition-screen

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

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

Batch checking

This screen shows a summary of all checked disposables, samples and reagents in one informational screen. Please make sure that all required components are loaded correctly. In case of any error, the problem will be highlighted in by a red font. If no errors during the loading steps occurred, proceed by pressing the button “Batch processing”.

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

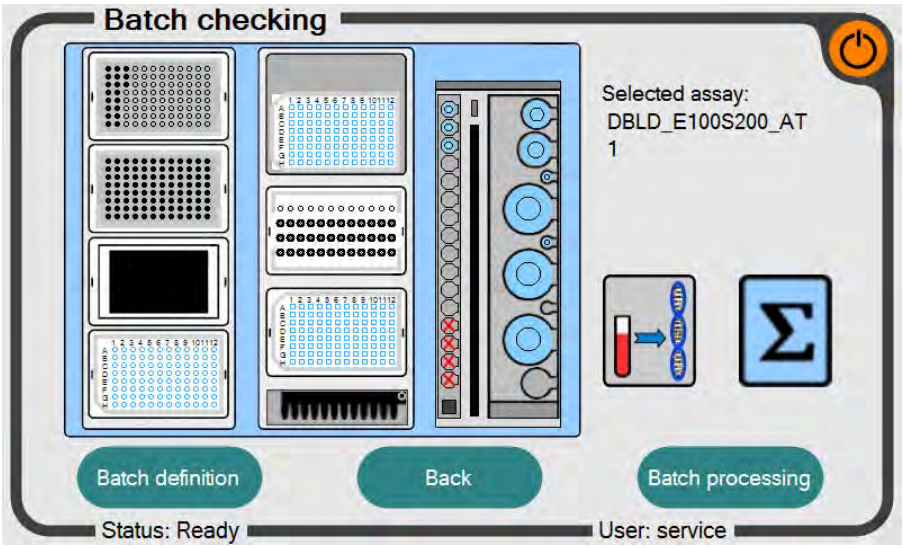


Figure 13: Batch-definition-screen

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 that requires user interaction. Do not try to force open the door during a run. This will cause an abort of the run!

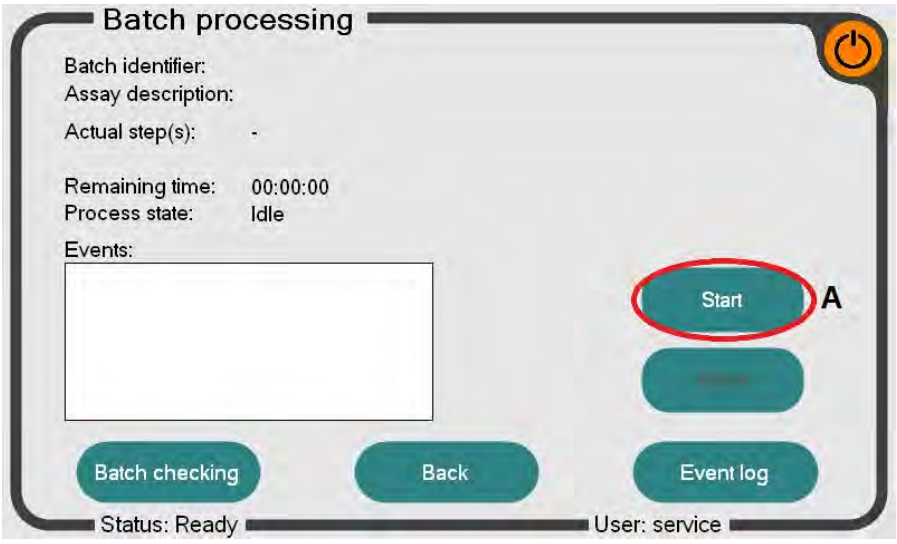


Figure 14: Batch-processing-screen

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

Note: ***The complete process will take approximately 90 minutes.***

After a run

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

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

Daily maintenance (UV decontamination)

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

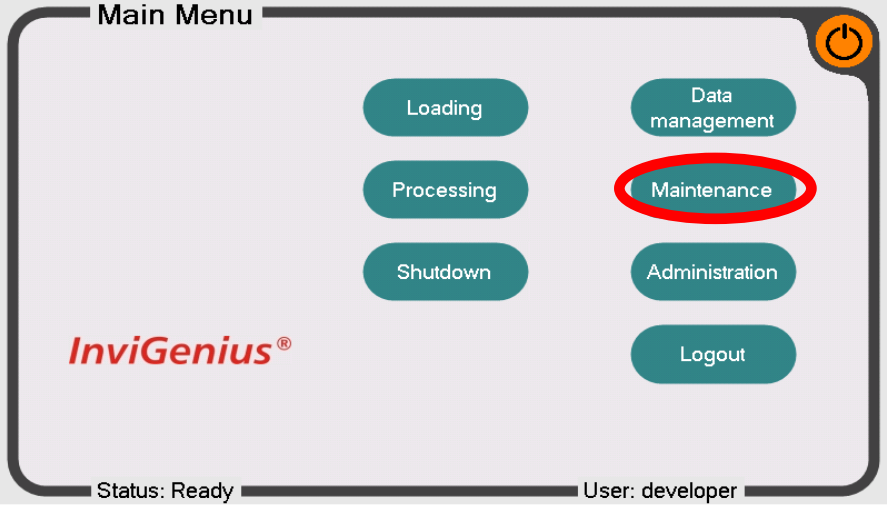


Figure 15: Main screen of the InviGenius® software

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

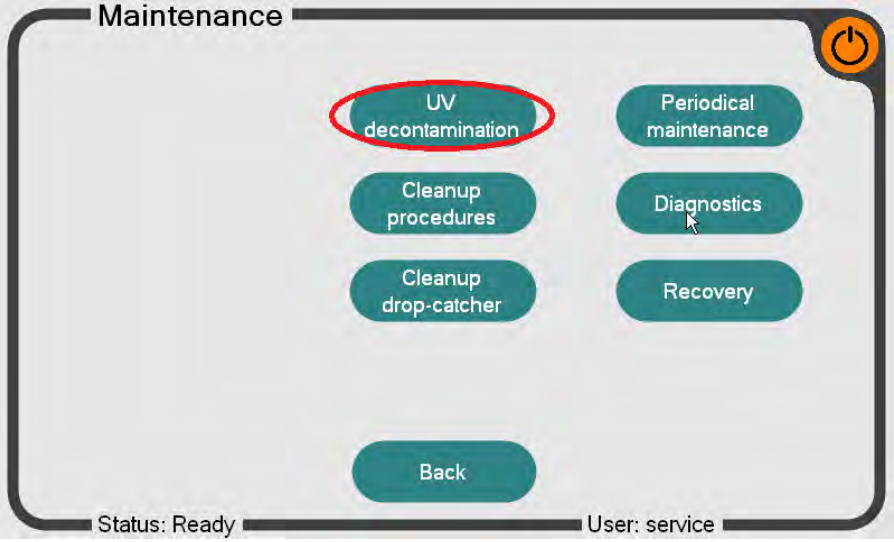


Figure 16: Maintenance screen of the InviGenius® software

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

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

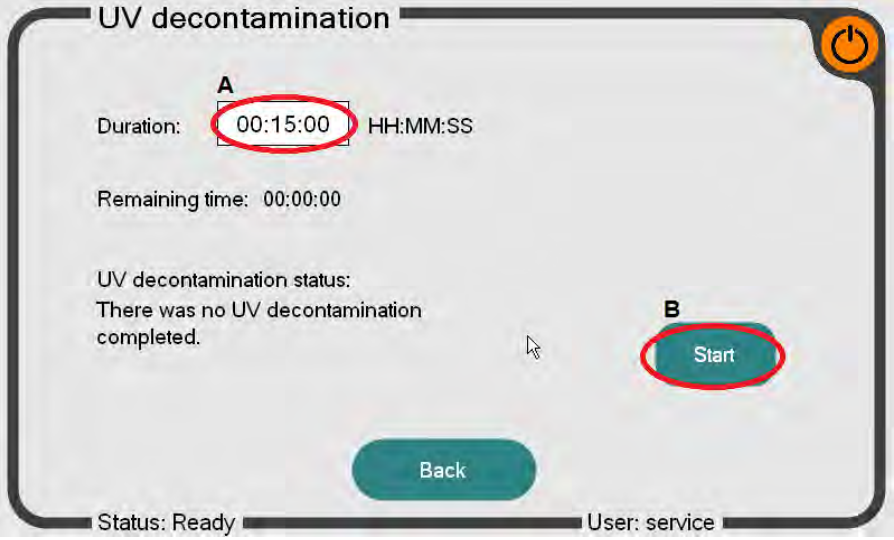


Figure 17: UV decontamination screen

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

Appendix

Example data

The below shown gDNA was derived with the InviMag® Blood DNA Mini Kit/ IG (200 µl whole 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

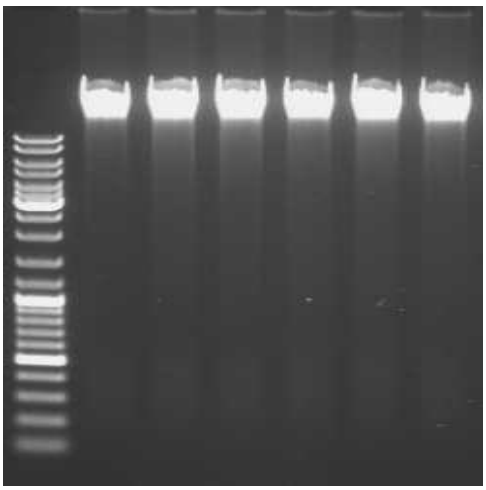
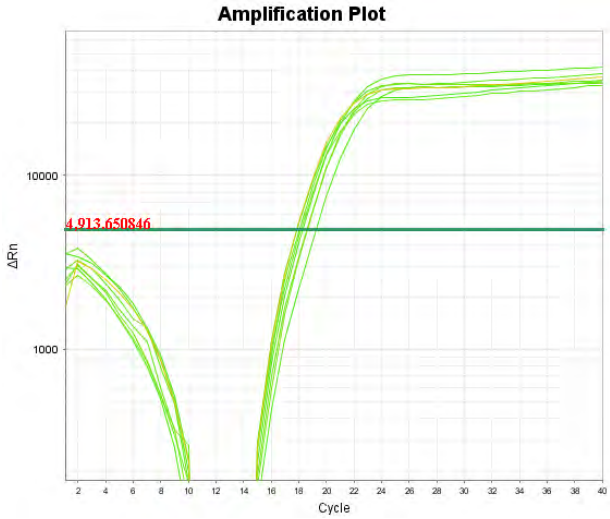


Figure B



The average purity (OD_{260}/OD_{280}) of all isolated samples was about 1.7 ± 0.1 and represents pure DNA. The overall yield of all samples was about 6 ± 0.4 µg. The elution volume was 200 µl. The photometric measurement was performed on a Nanodrop 1000 instrument using 2 µl sample whereas 10 µl of the respective eluates were used for gel electrophoresis (Fig.A) .

The performed PCR proved that no inhibition occurred. All samples were fully functional and showed a consistent amplification picture. The PCR was performed on a StepOnePlus cycler from Applied Biosystems in which 2 µl sample were analyzed.

The C_T values derived by the PCR, illustrated in Fig.B, demonstrate that all C_T values are in the same range with a very low standard deviation.

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 stored as written on page 8 sampling and storage of starting material. This procedure minimizes degradation of crude DNA by limiting the activity of endogenous nucleases.

Storage of DNA

Store genomic DNA in a fridge at 2-8 °C for max. 5 days, for long-term storage, store genomic DNA at -20°C. However, be careful because if the DNA is exposed to repeated freezing and thawing cycles this may cause shearing of the material.

Troubleshooting

Problem	Probable cause	Comments and suggestions
pipetting distribution errors	samples transfer failed / incomplete	the sample tube must contain at least 550 µl sample
	reagent / buffer transfer failed / incomplete	ensure that the supplied **Ethanol / Binding Solution** are filled up properly with either ethanol or isopropanol do not reuse bottles more often than described in Tab.1 because they will be rejected by the system
low concentration of extracted DNA	blood components settled	in case of large sample volumes (>>1 ml) carefully premix the sample tube before inserting it into the sample rack, the best way is on a Rotator for half an hour.
degraded DNA	incorrect storage of starting material	ensure that the storage of starting material is correct avoid multiple freezing and thawing cycles of the material
	old material	ensure that the starting material is fresh or stored at appropriate conditions (for long time storage at –20°C)! avoid multiple thawing and freezing cycles of the material old material may contain degraded DNA
no assay selectable	combination of reagents from different kits / missing required reagent	ensure that only and all reagents belonging to one kit type are used. a combination of reagents belonging to different kit types is not supported
eluted nucleic acids are brownish colored	residual magnetic particles are left in eluate	centrifuge the eluate plate at full speed for 1 min and transfer supernatant to a new plate / tube

Ordering information

Product	Package size	Catalogue No.
InviMag® Blood DNA Mini Kit/ IG	8 x 12 preps	2431120100
Related products		
InviMag® Blood DNA Maxi Kit/ IG	8 x 12 preps	2431320100
Invisorb® Spin Blood Mini Kit	250 preparations	1031100300
InviGenius® and consumables		
InviGenius®	1 unit	5011100000
Starting Box I/ IG	1 box	2400110100
Sheath Box Conductive filter tips, 1 ml; 2 x 2 rack/ pack (384 pieces) 5 Waste Trays 120 sample tubes		
Sheath Bundle	10 x 48 pieces	5011100300
Sheaths	1000 pieces	5011100200
Conductive filter tips, 1100 µl	10 x 96 pieces	5011100400
Waste Tray/ IG	25 pieces	5011100100

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

0062431120 V-01-2020