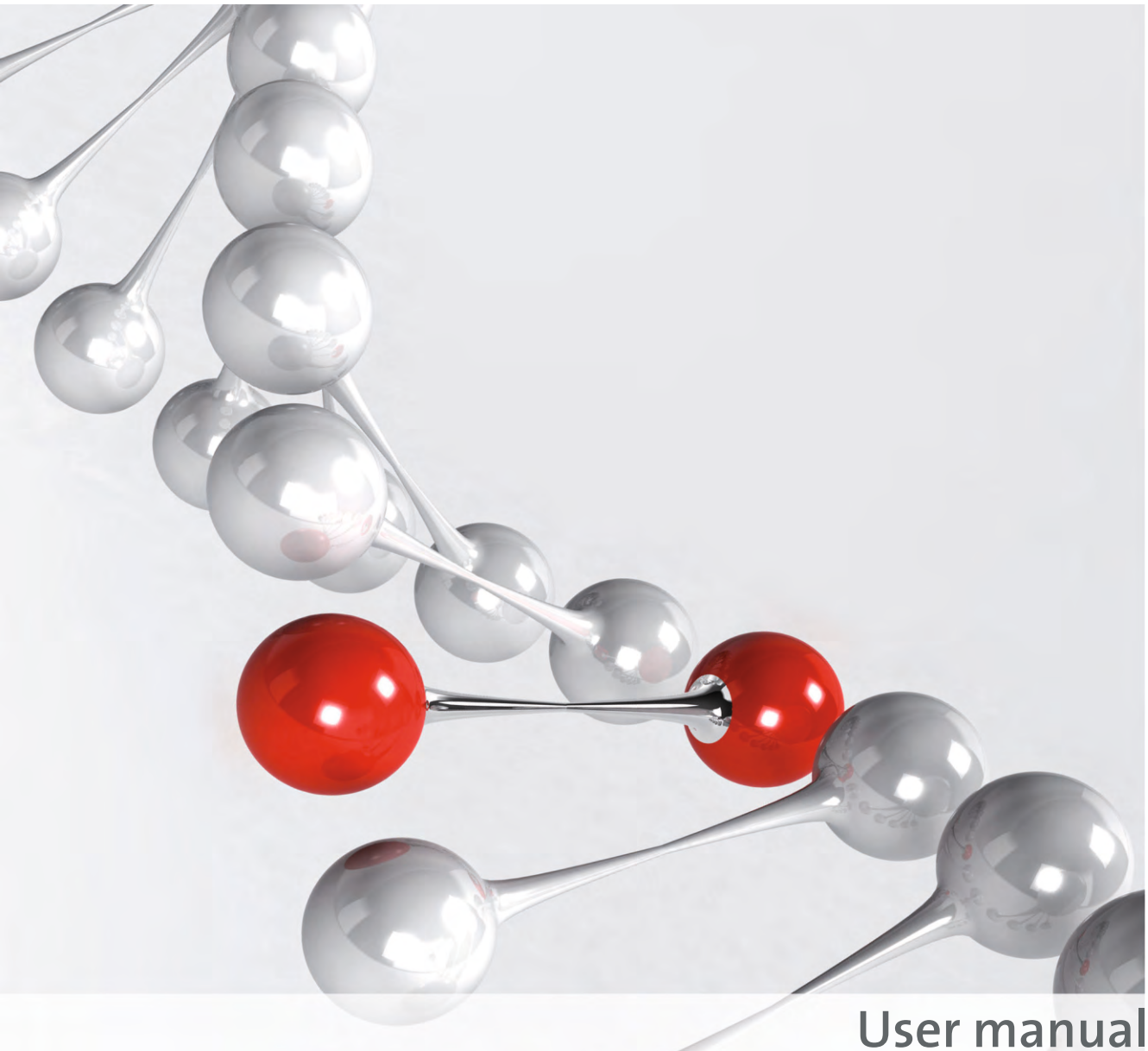
stratec
molecular



User manual
InviMag® SalivaGene DNA Kit /IG

for use on the InviGenius® and InviGenius® PLUS, STRATEC Molecular GmbH

for automated purification of total (genomic and mitochondrial) DNA from 1.6 ml stabilized saliva samples with magnetic beads

REF 2435260100 STRATEC Molecular GmbH, D-13125 Berlin

Instruction for InviMag® SalivaGene DNA Kit/ IG

The **InviMag® SalivaGene DNA Kit/ IG** is designed to quantitatively purify genomic DNA from saliva, which has been stabilized with a **SalivaGene Collection Set**. The purification process is accomplished by use of the patented InviMag® bead technology in combination with the InviGenius® platform from the STRATEC Molecular GmbH.

The **InviMag® SalivaGene DNA Kit/ IG** is the ideal tool for a walk-away automated isolation and purification of highly pure (genomic and mitochondrial) DNA from 1.6 ml of stabilized saliva samples. The kit is designed for an optimal use on the InviGenius® workstation. The interplay of the DNA extraction and purification chemistry provided by the **InviMag® SalivaGene DNA Kit/ IG** was intensely tested and validated.

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

The isolated DNA is ready to use for a broad panel downstream applications or can be stored at –20°C for subsequent use.

The kit is neither suitable for the isolation of DNA from stool samples, from fungi or bacteria nor for purification of total RNA.

For research use only!

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

The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

InviMag®, Invisorb® and InviGenius® are registered trademarks of STRATEC Biomedical AG.

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

© 2017 STRATEC Molecular, all rights reserved.

Contents

Kit contents of InviMag® SalivaGene DNA Kit/ IG

Component	4 x 12 reactions	8 x 12 reactions	Reagent sufficient for
Catalogue No.	2435260000	2435260100	
Proteinase K	For 4 x 1.8 ml working solution	For 8 x 1.8 ml working solution	1 run / tube
MAP Solution B	4 x 1.3 ml	8 x 1.3 ml	2 runs / tube
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 100 ml)	empty bottle (final volume 100 ml)	8 runs
Wash Buffer I	50 ml (final volume 105 ml)	50 ml (final volume 105 ml)	8 runs
Wash Buffer II	36 ml (final volume 126 ml)	2 x 36 ml (final volume 2 x 126 ml)	4 runs per bottle
Elution Buffer M	40 ml	2 x 40 ml	8 runs
Working Plate A	2	4	2 runs
Incubator Plate A	1	2	4 runs
Elution Plate E	1	1	8 runs
Microtube Cap	8	8	
Sheaths Box	1 (2 racks á 48 sheaths)	1 (2 racks á 48 sheaths)	4 runs / rack
Sealing Foils	4	4	
Incubator Stripe Foils	2	2	
Initial steps	Add 100 ml of 99.7% **Isopropanol** (molecular biologic grade) into each empty bottle Resuspend each lyophilized **Proteinase K** tube by addition of 1.8 ml dd-H_2O, and mix thoroughly! Add 55 ml of 96-100% ethanol to the bottle **Wash Buffer I**. Mix thoroughly and keep the bottle firmly closed! Add 90 ml of 96-100% ethanol to the bottle **Wash Buffer II**. Mix thoroughly and keep the bottle firmly closed!	Add 100 ml of 99.7% **Isopropanol** (molecular biologic grade) into each empty bottle Resuspend each lyophilized **Proteinase K** tube by addition of 1.8 ml dd-H_2O, and mix thoroughly! Add 55 ml of 96-100% ethanol to the bottle **Wash Buffer I**. Mix thoroughly and keep the bottle firmly closed! Add 90 ml of 96-100% ethanol to each bottle **Wash Buffer II**. Mix thoroughly and keep the bottle firmly closed!	

Content of SalivaGene Collection Sets

SalivaGene Collection Sets	50 pieces	8 x 50 pieces
SalivaGene Collection Module II	1035212200	1035212300
SalivaGene Collector	1035211200	

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® SalivaGene DNA Kit/ IG,** except **dissolved Proteinase K/ 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).

Proteinase K/ IG: Dissolved Proteinase K must be stored at 2 - 8 °C for up to two months. For longer storage –20 °C is recommended, freeze-thaw once only. So the dissolved Proteinase K is stable as indicated on the kit package.

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

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

Quality control and product warranty

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

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

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

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

For technical support or further information please contact:

from Germany: +49-(0)30-9489-2901/ 2910
from abroad: +49-(0)30-9489-2907 or contact your local distributor.

Intended use

The **InviMag® SalivaGene DNA Kit/ IG** is suitable for a reproducible fully automated extraction and purification of genomic DNA from 1.6 ml stabilized saliva samples. Up to 12 saliva samples can be processed using magnetic beads and the InviGenius® instrument. The DNA is suitable for almost every pharmacogenomics, forensic, human-identity, genetic or biosecurity analysis. The isolated DNA performs well in any downstream applications, such as quantitative PCR and STR analysis, with high signal-to-noise ratios.

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

The system consists of two modules, the collection module (**SalivaGene Collection Set**, which has to be ordered separately) and the extraction module (**InviMag® SalivaGene DNA Kit/ IG**).

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

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

Product use limitation

The kit is neither validated for the isolation of genomic DNA from serum, plasma, fungi, bacteria or parasites nor for isolation and purification of total RNA.

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 STRATEC Molecular product for any particular use. STRATEC Molecular does not provide validations of performance characteristics of the product with respect to specific applications.

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

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

All products sold by STRATEC Molecular are subject to extensive quality control procedures (according to EN ISO 9001 and EN ISO 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastics are for laboratory use only. They 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.stratec.com** for each STRATEC Molecular Product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

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

Below listed are European Community risk and safety phrases for the components of the **InviMag® SalivaGene DNA Kit/ IG**.

Wash Buffer I

Warning

H302-412-P280-P305-P351-P338-P273-EUH032

Proteinase K

Danger

H315-H319-H334-H335-P280-P305+P351+P338

Saliva DNA Stabilizer

Warning

H319-H412-P280-P305+P351+P338-P273

H302: Harmful if swallowed.
H315: Causes skin irritation.
H319: Causes serious eye irritation.
H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335: May cause respiratory irritation.
H412: Harmful to aquatic life with long lasting effects.
P273: Avoid release to the environment.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
EUH032: Contact with acids liberates very toxic gas.

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

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

Product characteristic of the InviMag® SalivaGene DNA Kit/ IG

The **InviMag® SalivaGene DNA Kit/ IG** is the ideal tool for an efficient and fully automated DNA extraction and purification from stabilized saliva samples using magnetic beads in combination with the **InviGenius®** robotic platform.

Starting Material	Typical Yield	Time	Ratio
1.6 ml stabilized saliva (SalivaGene Collector)	20-100 µg (depends on donor)	app. 120 min (depends on sample number)	A_{260} : A_{280} 1.5 – 1.8

The **InviMag® SalivaGene DNA Kit/ IG** in combination with a **SalivaGene Collection Set** uses the **PSP® Technology** (Preanalytical-Sample-Processing), a patented and cost efficient technology. The **SalivaGene System** is a modular system for collection, stabilization, storage and transportation of saliva samples using a **SalivaGene Collection Set** in combination with a defined and automated DNA purification procedure.

During collection, the 2 ml saliva sample is transferred into the **SalivaGene Collector** prefilled with lyophilized **SalivaGene DNA Stabilizer.** The raw material is lysed at specific conditions, including a quick inactivation of DNAses (prevention of DNA degradation), prelysis of the bacteria and inhibition of further bacterial growth. The barcode labeled tube of the **SalivaGene Collector** fits into the sample rack of the InviGenius®. The second module of the system, the **InviMag® SalivaGene DNA Kit/ IG** allows a very efficient and fast isolation of high quality genomic DNA from 1.6 ml stabilized saliva sample using the InviGenius® robotic platform from STRATEC Molecular.

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 performs 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 such as 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 reagent tracking, lot, shelf live, amount whereas a detailed inventory check helps to eliminate human errors, data storage, backup and archiving.

Due to the high purity, the isolated genomic DNA is ready-to-use in a broad panel of downstream applications (see below) or can alternatively be stored at –20°C for subsequent use.

- PCR*, real-time PCR*, RFLP / AFLP* analysis
- Restriction Enzyme Digestion
- HLA-Typing

*) The PCR method is covered by U.S. Patents 4,683,195 and 4,683,202 owned by Hoffmann-LaRoche Inc. The purchase of the **InviMag® SalivaGene DNA Kit/ IG** cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-LaRoche Inc.

Yield and quality of purified DNA

The amount of purified DNA depends on the sample source, transport, storage and age. Typically, a yield of 20 - 100 µg DNA is isolated from 1.6 ml of stabilized saliva.

The yield and quality of the purified genomic DNA is suitable for any molecular detection system. Any analytical test should be performed accordingly to the manufacturer's specifications.

Sampling and storage of starting material

Starting material

The amount of purified DNA using the **InviMag® SalivaGene DNA Kit/ IG** procedure can vary, depending on the amount of DNA present in the sample. The protocol works with a 1.6 ml aliquot of the transport media in which the saliva sample was collected and stored. The stabilized DNA is usable for up to one year if stored at room temperature and in the transport buffer (**SalivaGene DNA Stabilizer**). The aliquots of the stabilization buffer may be processed on the same day as collection or stored for future processing. For long-term storage freezing the sample at -20°C is recommended.

STRATEC 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® SalivaGene DNA Kit/ IG** procedure comprises the following steps:

- protein digestion of prelysed material in presence of **Proteinase K**
- binding of genomic DNA to magnetic beads (**MAP Solution B**)
- elimination of contaminants using **Wash Buffer I** and **Wash Buffer II**
- drying and elution of pure genomic DNA in **Elution Buffer M**

Lysis
Samples are lysed at non-chaotropic conditions at elevated temperature in stabilization buffer (**SalivaGene DNA Stabilizer**).

Protein digestion
Proteins are digested in presence of **Proteinase K**.

Binding genomic DNA
During addition of **Binding Solution** and **MAP Solution B** to the lysate, optimal binding conditions are adjusted and the genomic DNA is bound to the beads.

Removing residual contaminations
Contaminants are efficiently removed by using **Wash Buffer I** and **II**, while the genomic and mitochondrial DNA remain bound to the magnetic beads.

Elution of pure genomic DNA
After a drying step, the genomic DNA is finally eluted in **Elution Buffer M**. The eluted DNA is ready-to-use in different downstream applications.

Important notes

Important points before starting a protocol

Immediately upon arrival of the product, inspect the kit and its components as well as the package for any apparent visible damages and correct quantities. If there are any unconformities, please notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 6). Do not use damaged kit components because their use may lead to poor performance.

- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard contaminated gloves immediately
- Do not combine components from different kits
- Avoid microbial contaminations of the kit reagents.
- To minimize the risk of infections from potentially infectious sample materials, we recommend working under laminar air-flow when preparting the samples
- This kit should only be used by trained personnel.

Preparing reagents and buffers

Before performing a run, equilibrate all reagents at room temperature. If necessary, gently swirl and redissolve any precipitates by carefully warming up to 30°C. Try to avoid foaming.

- Add the required amount of distilled water to the Proteinase K containing tube and vortex the tube until the Proteinase K is completely dissolved (see page 3).
- Add the required amount of ethanol to the Wash Buffer I and Wash Buffer II bottles (see page 3)
- Add the required amount of isopropanol to the Binding Solution bottle.

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

4 x 12 Saliva DNA extractions:
Add 100 ml of 99.7% **Isopropanol** (molecular biologic grade) into each empty bottle Resuspend each lyophilized **Proteinase K** tube by addition of 1.8 ml dd-H_2O, and mix thoroughly! Add 55 ml of 96-100% ethanol to the bottle **Wash Buffer I**. Mix thoroughly and keep the bottle firmly closed! Add 90 ml of 96-100% ethanol to the bottle **Wash Buffer II**. Mix thoroughly and keep the bottle firmly closed!
8 x 12 Saliva DNA extractions:
Add 100 ml of 99.7% Isopropanol (molecular biologic grade) into each empty bottle Resuspend each lyophilized Proteinase K tube by addition of 1.8 ml dd-H2O, and mix thoroughly! Add 55 ml of 96-100% ethanol to the bottle Wash Buffer I. Mix thoroughly and keep the bottle firmly closed! Add 90 ml of 96-100% ethanol to each bottle Wash Buffer II. Mix thoroughly and keep the bottle firmly closed!

Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Conductive pipette tips (see ordering information, page 29)
- Disposable gloves
- PBS buffer
- ddH_2O
- Vortexer or rotator
- 96 - 100% ethanol
- Isopropanol*
- SalivaGene Collectors

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

Possible suppliers for Isopropanol:

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

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

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

Important indications

Minimum volume of samples in the SalivaGene Collection Tube
The procedure of the **InviMag® SalivaGene DNA Kit /IG** is optimized for the isolation of genomic DNA from up to 1.6 ml of saliva. We advise to provide at least 2 ml saliva per sample tube to prevent pipetting distribution errors during processing.

Sample volume smaller than 2 ml
For samples with a smaller volume than 2 ml, please adjust the **SalivaGene Collection Tube** with PBS or distilled water to a final volume of at least 2 ml.

Elution volume
The final elution volume is 500 µl. Typically, 500 µl eluate results in app. 20-100 µg DNA (depends on the donor).

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 risk is minimized.

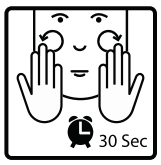
PSP®-Treatment with SalivaGene Collection Module II

Intended Use: Collection of human saliva samples for DNA stabilization and extraction (using the SalivaGene DNA Extraction Kits).

The DNA in SalivaGene stabilized samples is stable for 1 year at room temperature within the expiry date. The product is intended for use under supervision of professionals only, such as technicians, physicians and biologist trained in molecular biological techniques.

Contents: Collection tube, Stabilizer tube. The stabilizer tube contains approx. 2 ml of liquid Saliva DNA Stabilizer reagent.

Limitations of the procedure: Do not eat, drink, smoke or chew gum at least 30 min prior to saliva collection. Collecting 2 ml of saliva may require several minutes. Putting some grains of sugar on the tongue helps to produce saliva and does not interfere with the stabilization.

1. 	Unscrew lid from Collection Tube and put it aside for further use.
2. 	Rub cheeks against teeth intensely for 30 sec.
3. 	Collect saliva to indicated fill level, avoid making and measuring air bubbles.
4. 	Unscrew and discard lid from Stabilizer Tube. Pour Saliva DNA Stabilizer reagent into Collection Tube.
5. 	Screw lid tightly onto the Collection Tube again.
6. 	Shake tube for 15 sec to mix saliva and Saliva DNA Stabilizer reagent.

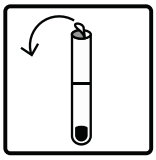
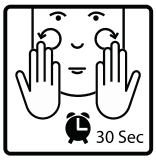
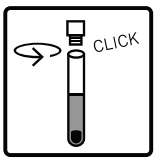
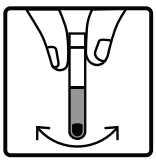
PSP®-Treatment with SalivaGene Collector

Intended Use: Collection of human saliva samples for DNA stabilization and extraction. Collection of human saliva samples for DNA stabilization and extraction (using the SalivaGene DNA Extraction Kits).

The DNA in SalivaGene stabilized samples is stable for 1 year at room temperature within the expiry date. The product is intended for use under supervision of professionals only, such as technicians, physicians and biologist trained in molecular biological techniques.

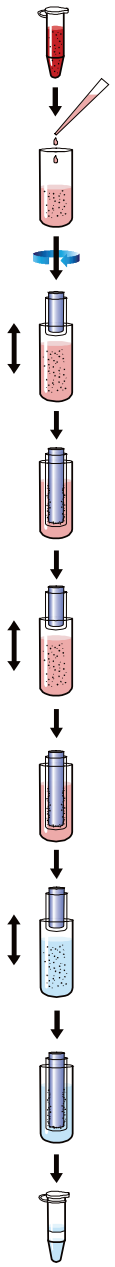
Contents: SalivaGene funnel, lid, tube, each tube contains approx. 150 mg of SalivaGene reagent.

Limitations of the procedure: Do not eat, drink, smoke or chew gum at least 30 min prior to saliva collection. Collecting 2 ml of saliva may require several minutes. Putting some grains of sugar on the tongue helps to produce saliva and does not interfere with the stabilization.

1. 	Remove seal from tube completely, and discard the seal or unscrew lid from tube and put it aside for further use.
2. 	Insert funnel into tube tightly.
3. 	Rub cheeks against teeth intensely for 30 sec.
4. 	Collect saliva to indicated fill level, avoid making and measuring air bubbles.
5. 	Remove and discard the funnel. Press lid firmly on the tube until it clicks or screw lid tightly onto the tube again.
6. 	Gently shake tube for 15 sec to dissolve white reagent.
7. 	Store the tube upright for 2-20 min with occasional shaking until SalivaGene reagent is dissolved. Some cloudy material may occur during this process. This does not interfere with stabilization.

Flow Chart of the InviMag® SalivaGene DNA Kit/ IG

Add the tubes of the SalivaGene Collectors in the sample loading rack.
Add the Buffers in the Buffer loading rack.



1.6 ml stabilized saliva sample is mixed with 120 µl **Proteinase K** at an elevated temperature (60°C)

After protein digestion, 800 µl **Binding Solution** and 80 µl **MAP Solution B** are added to the lysate

DNA binds to magnetic particles

Magnetic separation

Washing of the particle fixed DNA with:
1x 850 µl **Wash Buffer I**
2x 950 µl **Wash Buffer II**

Magnetic separation

Elution of DNA in 500 µl **Elution Buffer M.**

Magnetic separation and removal of **MAP Solution B**

Pure genomic DNA

Preparing the samples for processing on the InviGenius®

Please read the instructions carefully and conduct the prepared procedure.

Important Note: *The protocol is designed for the isolation of genomic DNA from 1.6 ml of saliva sample). To prevent possible distribution errors it is highly recommend using at least 2 ml of sample to ensure stable processing. If less than 2 ml sample is provided, please adjust the sample volume to 2 ml using either PBS or distilled water.*

1. Extraction of genomic DNA from stabilized saliva samples

If the saliva sample is freshly collected, we recommend incubating the tube for 1-2 hours at room temperature while shaking the SalivaGene Collector before starting with the purification process. If the sample was collected one or more days earlier, the purification process can be directly performed without any additional pretreatment. A good mixing of the sample with the lyophilized material can improve the yield (avoid foaming).

To use a sample tube in the InviGenius® platform, stick a patient barcode on the sample tube that is used for analysis. In case that no barcode is available, the sample identification name/number must be manually entered into the instrument during the loading process (see chapter “Sample loading”). No sample transfer to a new tube is required; the collection tube is directly used as a sample tube. A final sample volume of 1.6 ml saliva is processed by the InviGenius platform.

Note: *Do not forget to decap all tubes / reagent bottles before inserting them into the sample or reagent rack. Continue with the chapter “Preparing and loading of the InviGenius® system”.*

The required assay file stored in the instrument is “DSAL_E500_S1600” (explanation: DSAL = DNA from Saliva; E500 = Elution volume of 500 µl; S1600 = Sample volume of 1.6 ml)

General overview of the InviGenius® system

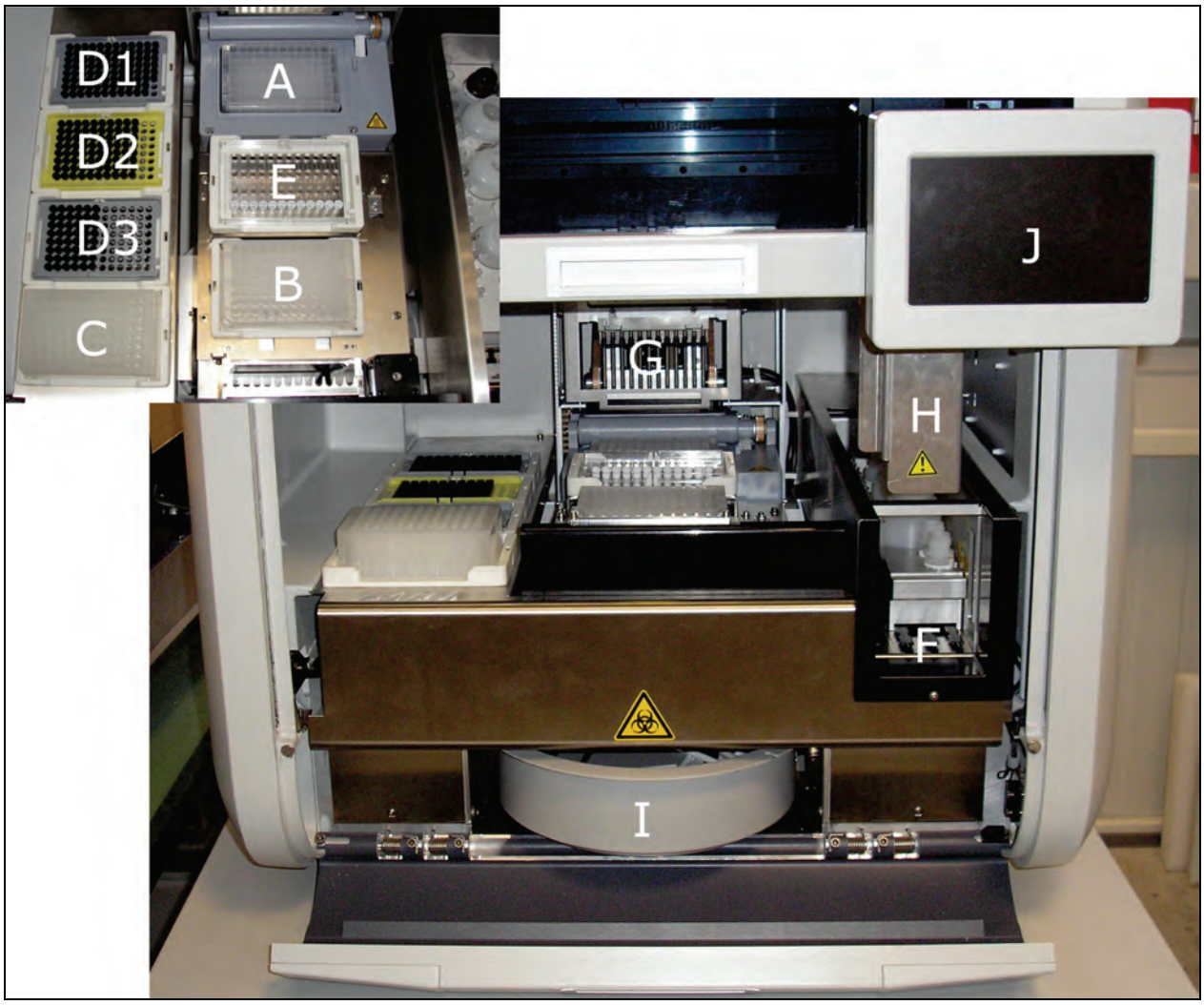


Figure 1: Frontal view of the InviGenius® System

There are three plate positions available in the InviGenius® system which can be loaded with corresponding plates: the incubator (A), working (B), and eluate position (C).

Lysis is performed at the incubator position (A), whereas the washing and elution process is performed at the working position (B). The eluate - containing the extracted nucleic acids – will be finally transferred to the eluate position.

Additionally, there are three loading positions available for disposable tip trays (D1-D3) and one 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 loaded into the right lanes 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 performed by use of the touch LCD (J) located at the top front right side.

Preparing and loading of the InviGenius®-system

Preparing the reagents

Before starting, dissolve one vial of Proteinase K with 1.8 ml of DNase-free water

Preparing the system

Turn on the InviGenius® system using the power switch located on the right back side of the instrument. The InviGenius® software is automatically launched during system start-up. Keep the door of the InviGenius® system closed during initialization.

After initialization of the InviGenius® system a login screen appears (Figure 2).

Log-in with the provided user name and password.

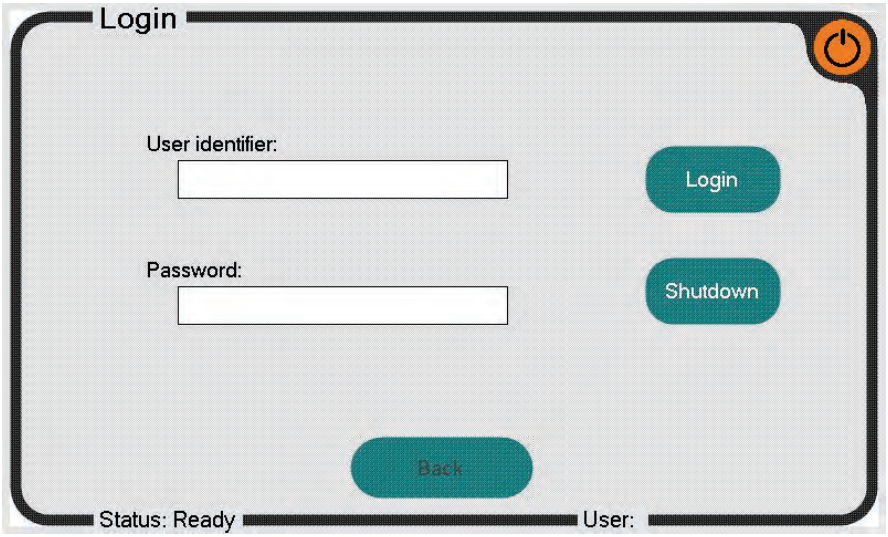


Figure 2: Login screen of the InviGenius® software

After login the main screen of the InviGenius® software is shown (Figure 3). Select “Loading” to start with loading of the system. Select “Processing” to define and run an assay if the system has already been loaded.

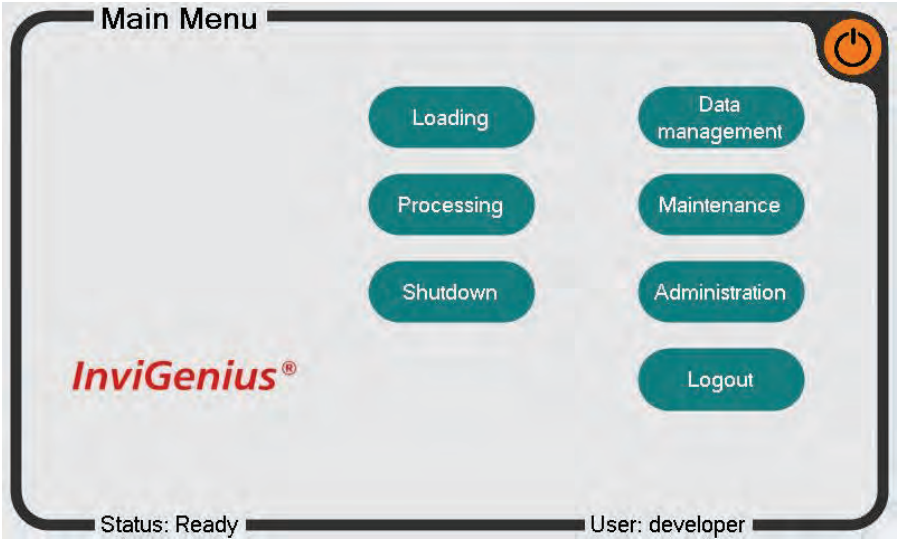


Figure 3: Main menu of the InviGenius® software

After selecting “Loading” the sample loading screen appears.

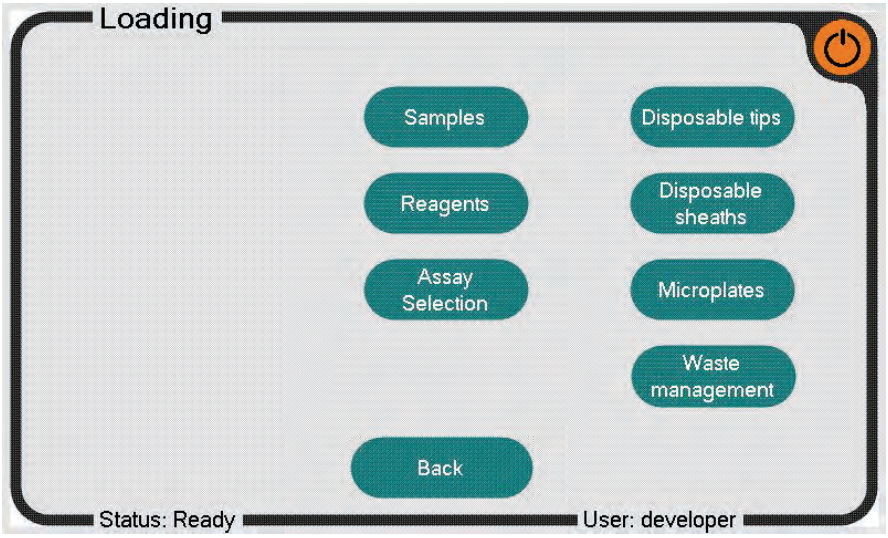


Figure 4: Loading screen of the InviGenius® software

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

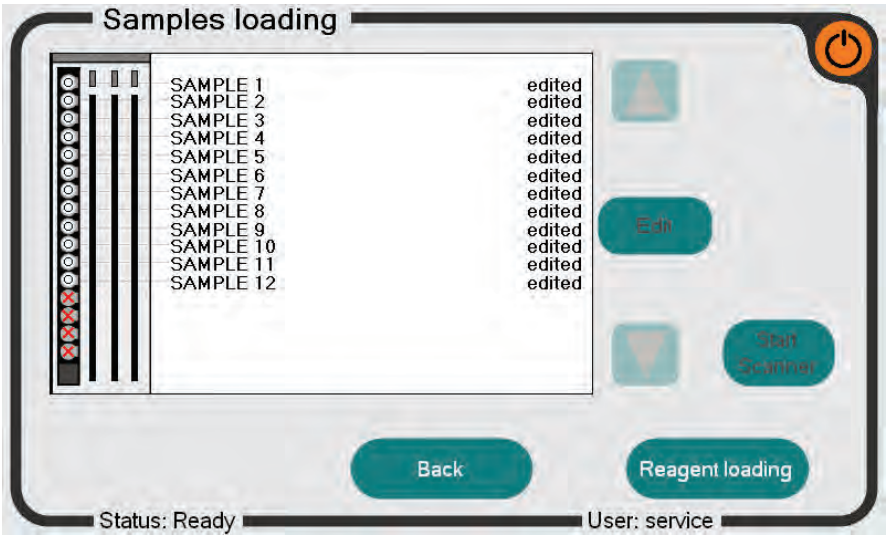


Figure 5: “Sample-loading” screen of the InviGenius® software

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

Please add the tubes of the SalivaGene Collector into the sample rack. For that purpose prefilled SalivaGene Collector or primary tubes prefilled with 2 ml sample from the SalivaGene Collection Module II can be used.

Please mix the collection tube to ensure a homogenized sample but avoid excessive foaming. For each reaction, a sample volume of 1.6 ml is processed. However, the total sample volume provided in the sample tubes must be ≥ 2 ml to ensure stable processing. Ensure that only the first 12 positions of the sample rack are used due to the limited number of wells per row of the plastic ware. For correct identification of a sample tube, the unique bar code (if available) must face to the bar code scanner located at the right side of the loading bay.

Please decap the tubes before transferring them into the loading rack.

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.

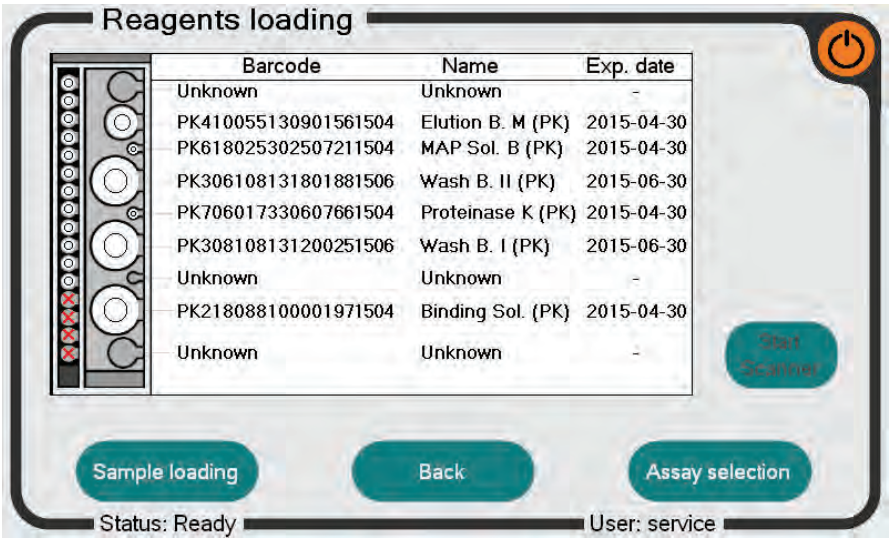


Figure 6: “Reagent loading” screen of the InviGenius software

Insert all provided reagents into the provided reagent rack of the InviGenius® system. Take care that the bar code labels face to the right side of the loading bay and decap the bottles and tubes. The order of the inserted reagents is not 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 repeat the procedure slowly.

Assay Selection

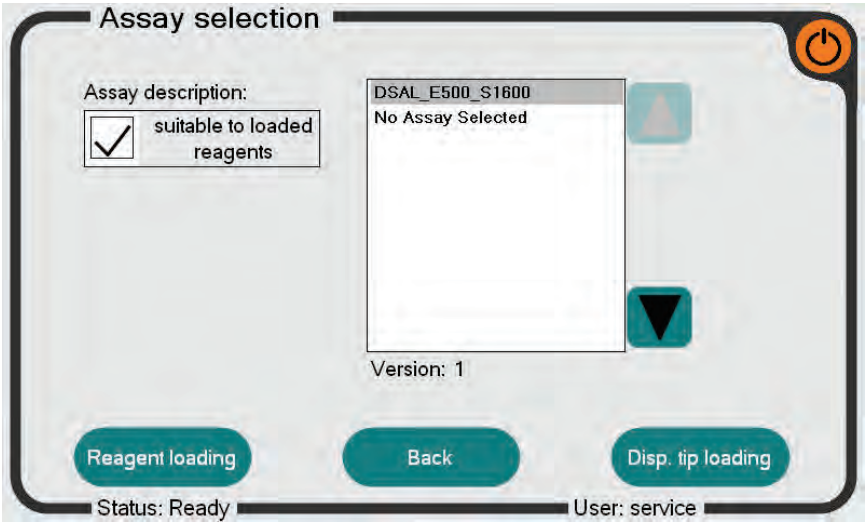


Figure 7: "Assay-selection" screen of the InviGenius software

Select the assay "DSAL_E500_S1600" and proceed with disposable tip loading. If no assay file is visible at least one reagent barcode was not recognized properly during the reagent loading procedure. Go back to Reagent Loading and repeat the loading process.

Disposable Tip Loading

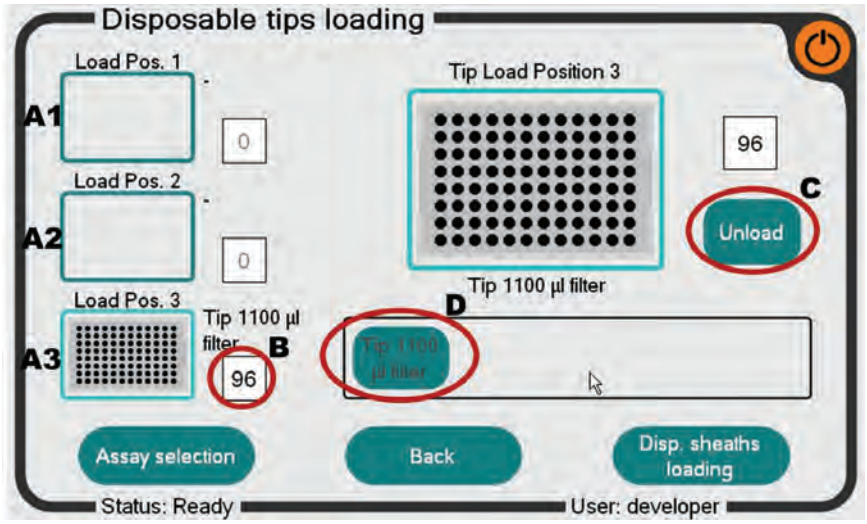


Figure 8: Disposable tip loading screen

There are three tip rack positions on the InviGenius® system (Fig. 8, A1-A3; corresponding to Fig. 3, D1-D3). 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 (D)

Each position can be filled either with 50 µl or 1100 µl filter or non-filter tips. However, for the SalivaGene assay, only 1100 µl filtered tips will be used.

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

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

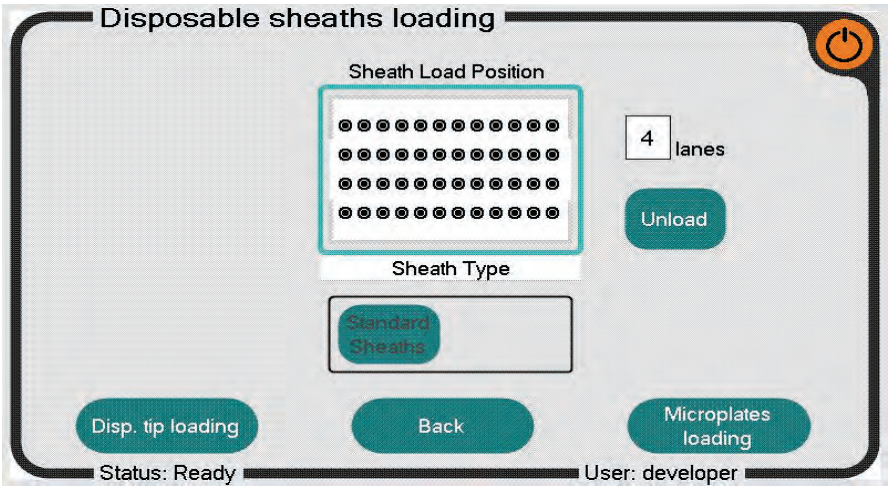


Figure 9: Disposable sheaths loading screen

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

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

Comparable to the disposable tips loading it is possible to define the number of rows left in the tip rack by pressing on the displayed number area. Make sure that the disposable sheaths are loaded (and displayed) consistent to the manually loaded sheaths in the rack to ensure correct sheaths pick up. Do not remove single disposable sheaths within a row of the sheaths rack if less than 12 samples are processed within one run 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 to not wash/reuse any disposed sheaths!

Plate Loading

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

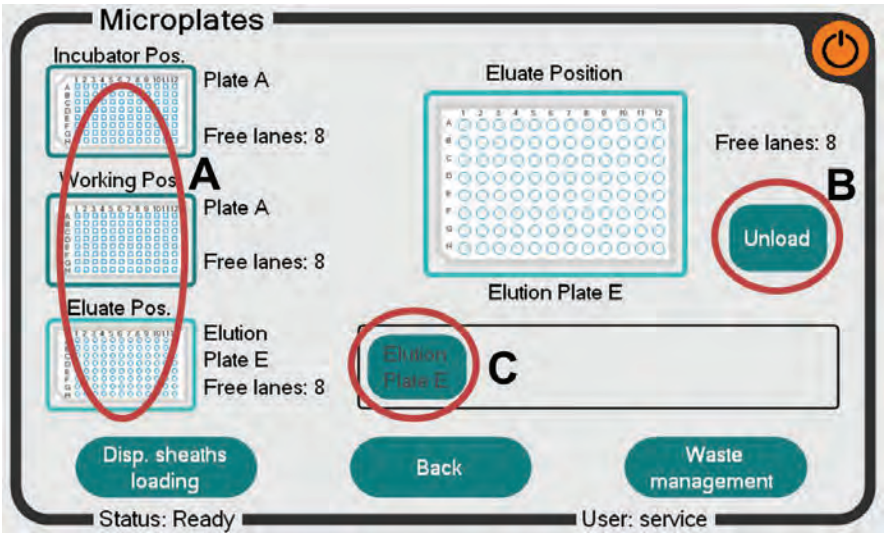


Figure 10: Plate loading screen

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.) A plate can be reloaded by pressing on the offered plate (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 ensure that the depicted lanes on the monitor are consistent with the real lanes in the corresponding positions.

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

Waste management

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

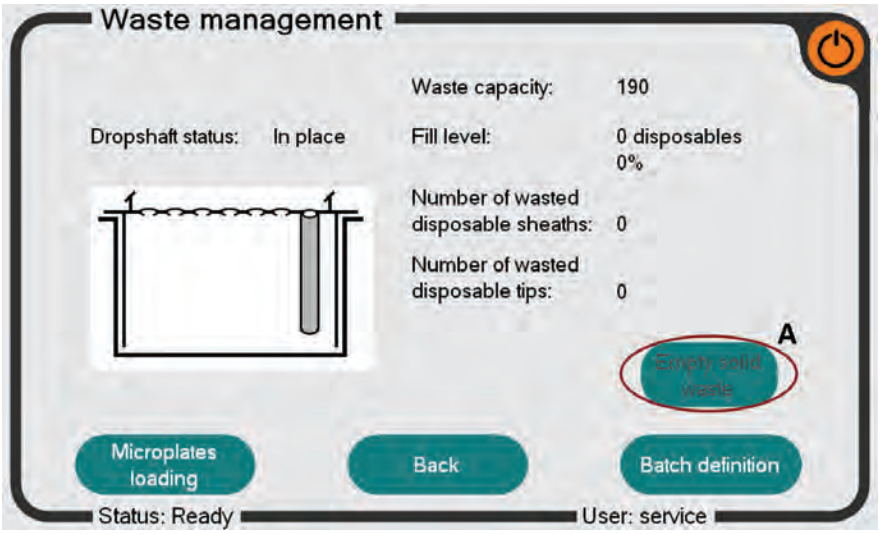


Figure 11: Waste-management-screen

If the waste tray was emptied, please use the “Empty solid waste” button (A).

Batch definition

Please select the appropriate assay (DSAL_E500_S1600) and check the loaded samples from which the DNA should be isolated.The assay can be selected by using the two arrow buttons (A). By default, all loaded and allocated samples are selected.

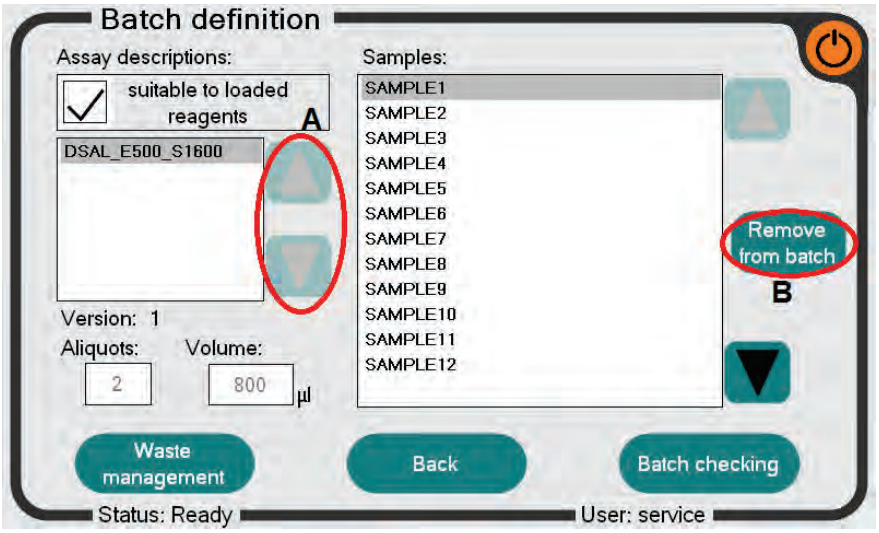


Figure 12: Batch definition screen

In principal, it is possible to change between assays, which are running with the appropriate buffers, tips and plates provided with the two arrow buttons (A). However, there is only one assay file available for the purification of saliva samples.
It is also possible to exclude/add sample(s) from the batch at this step. This can be done by excluding/adding the samples with the “Remove from batch”/”Adding to batch” button (B). Use the arrow buttons to switch between the samples.

Batch checking

In this screen all loaded disposables, samples and buffers are checked and summarized in one info screen. Please ensure that the system has been loaded properly. Any error will be elucidated in red font illustrated on the display. If the system is ready to proceed and - no error is detected / no red highlighted area is visible - continue by pressing the button “Batch processing”. If an error is present, it can be solved by directly clicking into the red highlighted area. A new screen will appear showing instructions (printed on the screen) how to solve the problem. Follow the instructions to fix the corresponding error status. Continue with “Batch processing”.

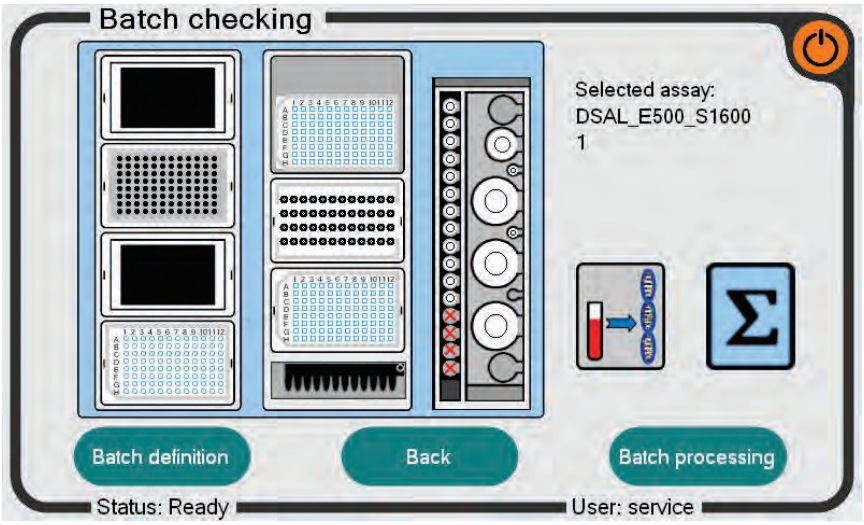


Figure 13: Batch definition screen

Batch processing

After closing the system door, the assay can be started by pressing the “Start process” button (A). The door will be locked throughout the whole purification process and the system will start with sample processing. Do not forget to close or try to force open the locked door. This will lead to an error resulting in an abort of the run.

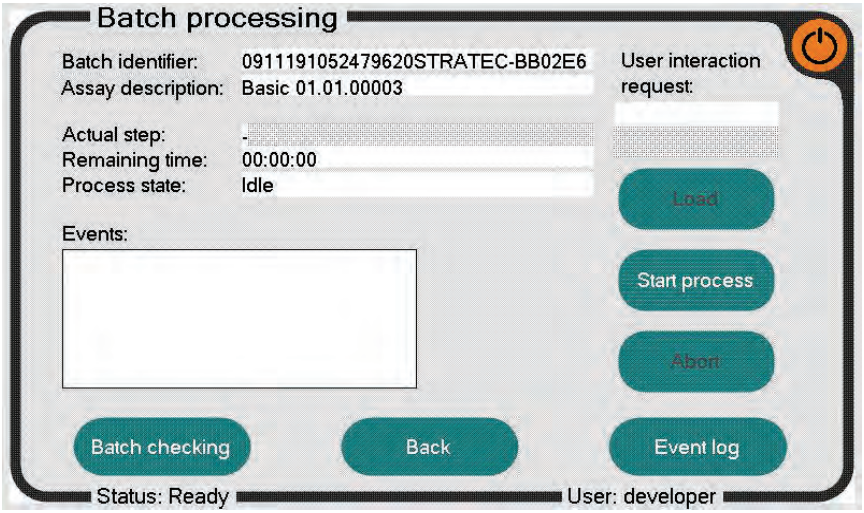


Figure 14: Batch processing screen

At the end of the process, the genomic DNA containing eluates are located in the appropriate cavities of the eluate plate and can be used for further applications.

After the run

After the run is finished, please unload (**do not** erase the plate status yet if the should be used later) all used plates and reagents and store them according to GLP-guidelines. Please keep in mind that the plates could contain possibly pathogenous material.

Important: *Do not reset the plate allocation if the plate(s) should be reused later. After the plate allocation information is deleted, there is no possibility to add/delete free/used lane positions of a plate. This means the previously used plate has to be discarded.*

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

Note: *We recommend decontaminating the instrument at the end of each working day by using the internal UV lamp. In case of liquid spilling inside the instrument, please clean contaminated areas by using ethanol or soap water. Do not use ethanol on plastic parts like the front cover. This can lead to permanent clouding of affected parts.*

Daily maintenance (UV decontamination)

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

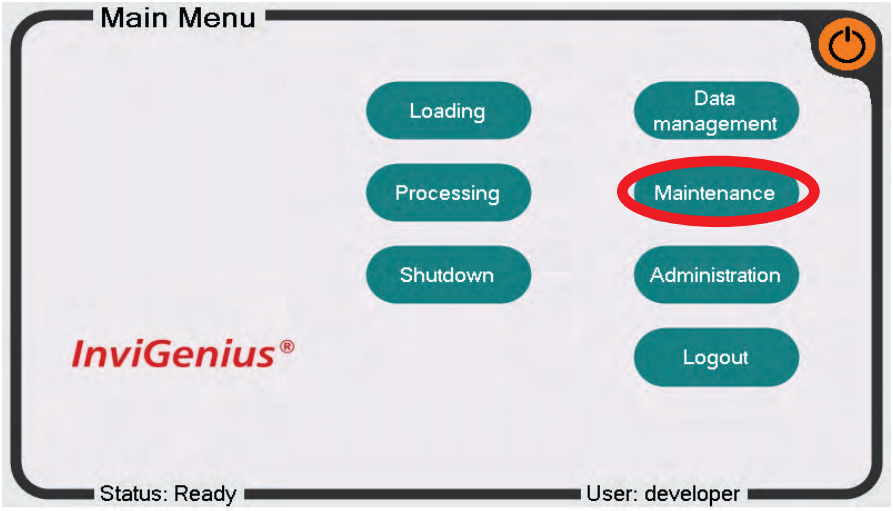


Figure 15: Main screen of the InviGenius® software

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

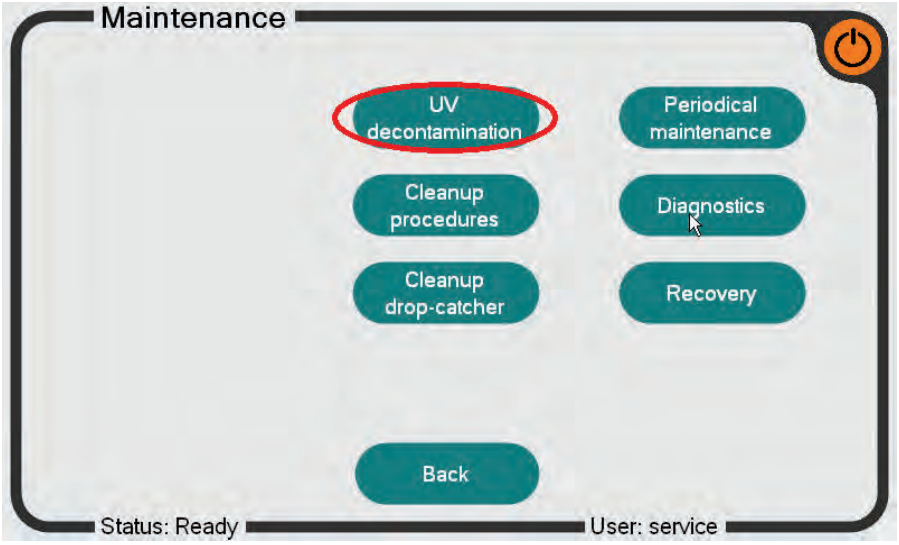


Figure 16: Maintenance screen of the InviGenius® software

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

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

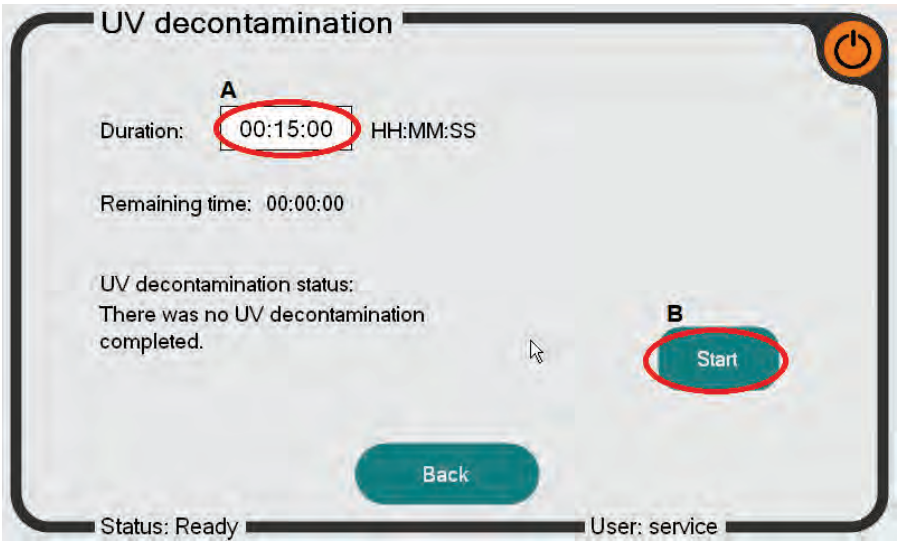


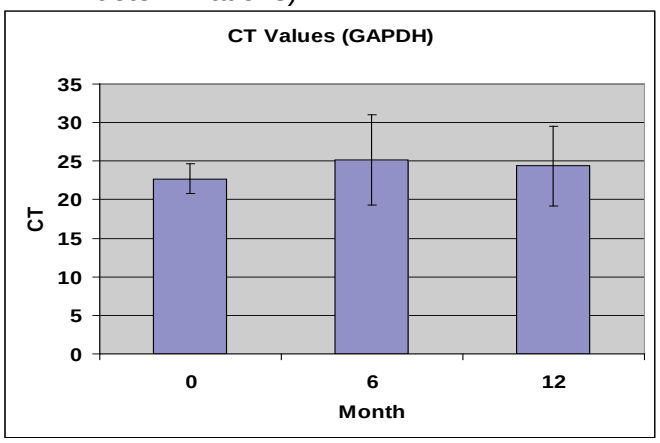
Figure 17: UV decontamination screen

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

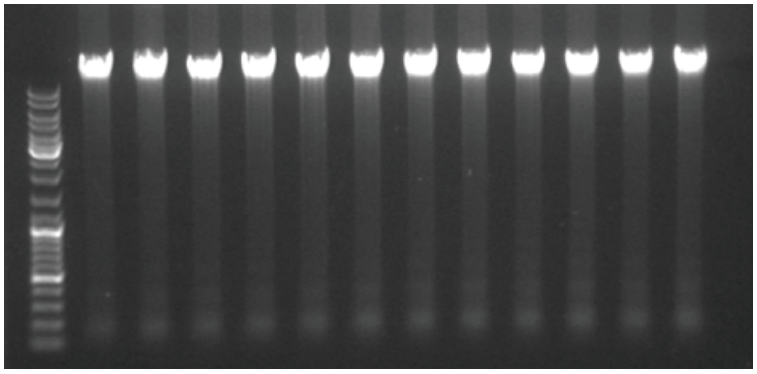
Appendix

Example data

(A) Mean CT values of real-time PCR reactions using primers for GAPDH of DNA aliquots from 200 µl of saliva after 0, 6 and 12 months of storage in PSP SalivaGene stabilization buffer at room temperature (average of 10 different samples, single determinations).



Genomic DNA was purified on the InviGenius® from 1.6 ml of stabilized saliva samples (pooled samples of ten different donors). 10 µl of the eluate were loaded on a 0.8% agarose gel stained with ethidium bromide.



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	Transfer of **Proteinase K** failed	Ensure that lyophilized **Proteinase K** is resuspended with the appropriate volume of water
	Samples transfer failed	The sample tube must contain at least 2.0 ml sample
	Reagent / buffer transfer failed / incomplete	Ensure that the **Wash Buffer** and **Binding Buffer** is filled up properly with ethanol/isopropanol Do not reuse bottles more often than described in Tab.1
Low concentration of extracted DNA	Sample 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 / isopropanol added to **Wash Buffer / Binding Buffer**	Assure that the **Wash Buffer** / **Binding Buffer** is filled up with ethanol / isopropanol properly as indicated in Tab. 1
Degraded or sheared DNA	Incorrect storage of starting material	Ensure that the storage condition of the starting material was correct Avoid multiple freezing and thawing cycles of the sample
	Old material	Ensure that the starting material is fresh or stored at appropriate conditions (for long-term storage at -20°C) Old material often contains degraded DNA
No assay selectable	Combination of reagents from different kits or blocked barcode during reagent loading procedure	Assure that only reagents belonging to one kit type are used. a combination of reagents belonging to different kit types is not supported by the system Ensure that the reagent barcode labels are visible within the reagent rack reading frame
Eluted DNA is brownish colored	Small part of the magnetic particles are left in the elution	Centrifuge the eluates at full speed for 1 min and transfer supernatant to a new plate / tube

Ordering information

Product	Package size	Catalogue No.
InviMag® SalivaGene DNA Kit/ IG	8 x 12 preps	2435260100

Related products	Package size	Catalogue No.
PSP® SalivaGene DNA Kit	50 preparations	1035200200
PSP® SalivaGene DNA Kit	250 preparations	1035200300
InviMag® SalivaGene DNA Kit /KF96	1x 96 preps	7435300100
InviMag® SalivaGene DNA Kit /KF96	5x 96 preps	7435300200
SalivaGene® Collection Module II	10 x 50 container	1035210800
SalivaGene® Buccal Swab	10 x 50 pieces	1035230300
SalivaGene DNA Stabilizer	30 ml	1035201100

InviGenius® and consumables

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

Possible suppliers for Isopropanol:

Carl Roth
2-Propanol
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

stratec molecular

STRATEC Molecular GmbH
Robert-Rössle-Str. 10
13125 Berlin, Germany

Phone: +49 30 94 89 29 01
Fax: +49 30 94 89 29 09
E-mail: info.berlin@stratec.com

www.stratec.com

1EG38/IC/03/2017