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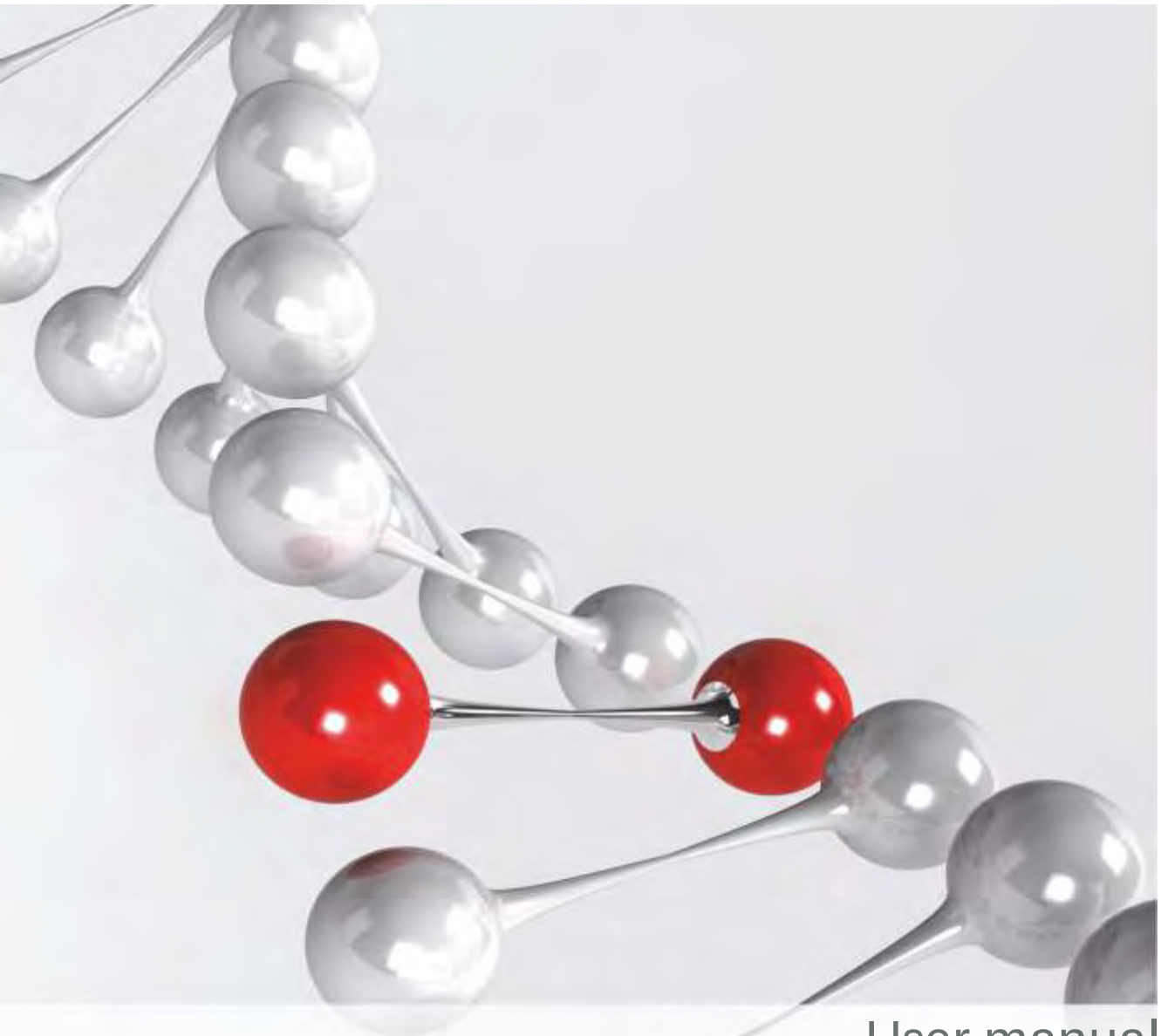

User manual
InviMag® SalivaGene DNA Kit/ KF96

for use on KingFisher™ 96 and KingFisher™ Flex, Thermo Fisher Scientific

for automated purification of total DNA from SalivaGene stabilized clinical swab (mouth brushes) & saliva samples with magnetic beads

REF 7435060X00 STRATEC Molecular GmbH, D-13125 Berlin

Instruction for InviMag® SalivaGene DNA Kit/ KF96

The **InviMag® SalivaGene DNA Kit /KF96** is designed to purify genomic DNA from SalivaGene stabilized saliva and swab samples. The purification process is accomplished by use of the InviMag® magnetic bead technology in combination with the KingFisher™ KF96 or KFflex 96 instrument.
The **InviMag® SalivaGene DNA Kit /KF96** is a modular system using a stabilization solution for collection, stabilization, storage and transportation of saliva material without degradation of DNA.

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

Table of Content

Kit content of the InviMag® SalivaGene DNA Kit/ KF96

	1x 96 extractions	5x 96 extractions
Catalogue No.	7435060100	7435060200
Binding Buffer A	9 ml (final volume 30 ml)	36 ml (final volume 120 ml)
Proteinase S	2 x 2 ml	6 x 2 ml
SNAP Solution	2 x 1.1 ml	10.5 ml
Wash Buffer I	30 ml (final volume 60 ml)	2 x 80 ml (final volume 2 x 160 ml)
Wash Buffer II	45 ml (final volume 150 ml)	3 x 60 ml (final volume 3 x 200 ml)
Elution Buffer	15 ml	60 ml
2.0 ml Deep Well Plate	4	20
KF 96 Tip Comb for DW magnets	1	5
200 µl Elution Plate*	2	10
Sealing Foils	2	10
Manual	1	1
Initial steps	Add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min.. Shortly before use mix by inverting several times. Add 30 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap at RT. Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap at RT.	Add 84 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min.. Shortly before use mix by inverting several times. Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap at RT. Add 140 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap at RT.

Kit content of the InviMag® SalivaGene DNA Kit/ KF96 w/o plastic

	1x 96 extractions	5x 96 extractions
Catalogue No.	7435060150	7435060250
Binding Buffer A	9 ml (final volume 30 ml)	36 ml (final volume 120 ml)
Proteinase S	for 2 x 2 ml working solution	6 x 2 ml
SNAP Solution	2 x 1.1 ml	10.5 ml
Wash Buffer I	30 ml (final volume 60 ml)	2 x 80 ml (final volume 2 x 160 ml)
Wash Buffer II	45 ml (final volume 150 ml)	3 x 60 ml (final volume 3 x 200 ml)
Elution Buffer	15 ml	60 ml
Sealing Foils	2	10
Manual	1	1
Initial steps	Add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min.. Shortly before use mix by inverting several times. Add 30 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap at RT. Add 105 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap at RT.	Add 84 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min.. Shortly before use mix by inverting several times. Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap at RT. Add 140 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap at RT.
Plastic to be supplied by user (see order information)		
KF 96 Tip Comb for DW magnets	1	5
200 µl Elution Plate*	2	10
2.0 ml Deep Well Plate	4	20

Content of SalivaGene Collection Sets

SalivaGene Collection Sets	50 pieces	
SalivaGene Collection Module II	1035212200	1035212300 (125 pieces)
SalivaGene Collector	1035211200	
SalivaGene Swab Comfort	1035231200	1035231300 (300 pieces)

Symbols


Manufacturer

Lot number

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

Catalogue number


Expiry date


Consult operating instructions


Temperature limitation


Do not reuse


Humidity limitation

Storage

All buffers and kit contents of the **InviMag® SalivaGene DNA Kit/ KF96** 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).

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

Binding Buffer charged with 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/ KF96** for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, 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 13485 certified Quality Management System the performance of all components of the **InviMag® SalivaGene DNA Kit/ KF96** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **InviMag® SalivaGene DNA Kit/ KF96** 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 at 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/ KF96** is suitable for a reproducible purification of total DNA from up to 500 µl stabilization media of saliva or swab samples in forensic, human-identity, genetic, biosecurity and pathogen analyses. The purification is very efficient and the isolated DNA performs well in downstream applications, such as quantitative PCR and STR analysis, with high signal-to-noise ratios.

The system consists of two modules, one collection set and one extraction module, useable independently or in combination.

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

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

Product use limitation

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

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide for validation 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 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 plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The product and its content are unfit 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.molecular.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/ KF96** 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.

European Community risks and safety phrases for the components of the **InviMag® SalivaGene DNA Kit/ KF96** to which they apply are listed below as follows:.

Wash Buffer I

Warning

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

Proteinase S

Danger

H317-H318-P280-P305+P351+P338

Saliva DNA Stabilizer

Warning

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

H302: Harmful if swallowed.
H317: May cause an allergic skin reaction.
H318: Causes serious eye damage.
H319: Causes serious eye 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/ KF96

Starting material	Yield	Time	Ratio
500 µl of stabilization buffer (SalivaGene DNA Stabilizer) for **SalivaGene Collector/ SalivaGene Collection Module II** or 500 µl of swab stabilization (SalivaGene DNA Stabilizer) for **Salivagene Swab Comfort Set**	up to 10 µg (depends on starting material) up to 1 µg (depends on the donor)	about 60 min (depends on the used protocol)	A_{260} : A_{280} 1.6 – 2.1 Low DNA contents may result in values > 2,1!

The **InviMag® SalivaGene DNA Kit/ KF96** uses the **PSP® Technology** (Preanalytical-Sample-Processing) a patented cost efficient technology and is a modular designed system for collection, stabilization, storage and transportation of saliva samples with DNA purification. The system combines the use of a prefilled **SalivaGene Collection Tube** for saliva or swab sample collection, the storage and stabilization of saliva specimen without any degradation of the DNA during transportation, including the prelysis of bacteria and a very efficient and fast isolation (about 60 min) of high quality total DNA from up to 500 µl sample mixture. The kit is designed for isolation of DNA from host organism as well as for isolation of DNA from pathogen microorganisms.

After collection, the sample is transferred to the **SalivaGene Collection Tube** prefilled with **SalivaGene DNA Stabilizer.** The **SalivaGene DNA Stabilizer** leads to an inactivation of DNases and prevents the degradation of the DNA, preserves the microorganism titer and prelyses bacteria. The transport of the stabilized DNA can be carried out in the **SalivaGene DNA Stabilizer** containing tubes without cooling. For the DNA extraction process an aliquot or the whole volume is used. The residual sample can be stored and used for further extractions.

A 500 µl aliquot of the stabilized sample is used in a **PSP® SalivaGene DNA** isolation procedure. At first, the proteins are degraded with Proteinase S, followed by binding of the DNA to magnetic beads while contaminants, like potent inhibitors and proteins, are rejected and removed leaving the pure DNA to be eluted in Elution Buffer. Yields may be varying from sample to sample depending on factors such as the health of the donor, , or sample storage conditions.

The procedure neither requires a phenol / chloroform extraction nor an alcohol precipitation. The method requires only minimal user interaction allowing safe handling of potentially infectious samples. The procedure is designed to avoid sample-to-sample cross-contamination.

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

- PCR*, real-time PCR*, RFLP / AFLP*- analysis
- restriction enzyme digestion
- Hybridisation
- Sequencing

Sampling and sample storage

Starting material

The amount of starting material used in the **InviMag® SalivaGene DNA Kit/ KF96** procedure can vary, depending on the amount of DNA present in the sample. Specific guidance for starting amounts is given in the individual protocols.

Saliva:

The protocols work with aliquots of transport media for collection of fresh saliva or swab samples. The DNA of fresh saliva (collected using the **SalivaGene Collector** or **SalivaGene Collection Module II**) is stable for at least one year at room temperature in the stabilization buffer. The sample can alternatively be stored in this buffer at -20°C for long-term storage.

Swab:

The DNA of swab samples (collected using the **SalivaGene Swab Comfort**) is stable for at least 6 months at room temperature in the stabilization buffer. Long-term storage (≥ 6 month) of the sample should be done at - 20°C after receiving the collection device. Please, before freezing the sample squeeze and remove the swab.

STRATEC Molecular will be released of its responsibilities, if other sample materials than described in the Intended Use are processed or if the sample preparation protocols are changed or modified.

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Principle and procedure

The **InviMag® SalivaGene DNA Kit/ KF96** procedure comprises following steps:

- collection of the material using a **SalivaGene Collection Set**
- lysis of cells in presence of non-chaotropic conditions at elevated temperature in the Stabilization Buffer (**SalivaGene DNA Stabilizer**)
- protein digestion (**Proteinase S**)
- binding of the genomic DNA to magnetic beads (**SNAP Solution**)
- washing of the beads and elimination of contaminants and ethanol
- elution of pure genomic or bacterial DNA

This manual contains two protocols, according to the different requirements of the starting materials and the extraction instrument used.

Lysis
Samples are lysed in presence of non-chaotropic conditions at elevated temperature in the Stabilization Buffer (**SalivaGene DNA Stabilizer**).

Protein digestion
Proteins are digested in the lysed samples in the presence of **Proteinase S**.

Binding genomic DNA
By adding **Binding Buffer A** to the lysate, optimal binding conditions are adjusted.
Each lysate is then mixed with magnetic beads and the genomic DNA is bound to the beads.

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

Elution of pure genomic DNA
The nucleic acids are eluted from the magnetic beads using **Elution Buffer** and are ready-to-use in different downstream applications.

Yield and quality of DNA

The amount of purified DNA in the **InviMag® SalivaGene DNA Kit/ KF96** procedure from different samples depends on the sample type and sample source,. Yield and quality of isolated DNA are suitable for any downstream detection system. The diagnostic tests should be performed accordingly to manufacturer's specifications.

Important points before starting a protocol

General handling notes

Immediately upon receipt of the product, inspect the product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify 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 7). Do not use damaged kit components because their use may lead to poor kit performance.

- Always change pipette tips between different liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipette tips.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Discard contaminated gloves immediately.
- Do not combine components of different kits, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar airflow until the samples are lysed.
- 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 re-dissolve any precipitates by warming up to 30°C. Swirl gently to avoid foaming.

Add the required amount of ethanol to **Wash Buffer I** and **Wash Buffer II** and Isopropanol to **Binding Buffer A**
Elution Buffer is ready-to-use.

1x 96 DNA extractions:
Add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.
Add 30 ml of 96-100% Ethanol to the bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap at RT.
Add 105 ml of 96-100% Ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap at RT.

5x 96 DNA extractions:
Add 84 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.
Add 80 ml of 96-100% Ethanol to each bottle **Wash Buffer I**, mix thoroughly and store with tightly closed cap at RT.
Add 140 ml of 96-100% Ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap at RT.

Reagents and equipment to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.molecular.stratec.com** under each STRATEC Molecular kit and kit component.

- Measuring cylinder (250 ml)
- Disposable gloves
- Pipette and pipette tips
- Vortexer
- ddH_2O
- 96-100% Ethanol
- Isopropanol*

*The **InviMag® SalivaGene DNA Kit/ KF96** 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

- Samples and buffers should be thoroughly mixed and should have room temperature.
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- The volume of the eluted DNA may be lower than the added volume of **Elution Buffer**.
- The **Elution Buffer** doesn't contain EDTA.

PSP®-Treatment with SalivaGene Collector

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

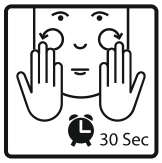
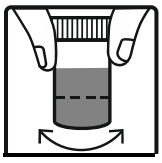
Limitations of the procedure:
Do not eat, drink, smoke or chew gum at least 30 min prior to saliva collection. Collecting 2 ml of saliva may require several minutes. Putting some grains of sugar on the tongue helps to produce saliva and does not interfere with the stabilization.

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. 	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.
8. 	For barcode sample tracking stick the small barcode tape vertically onto the tube.

PSP®-Treatment with SalivaGene Collection Module II

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 of SalivaGene Swab Comfort

Contents:
Swab, Stabilizer tube, label for donor description. The Stabilizer tube contains approx.650 µl of liquid DNA Stabilizer reagent.

Limitations of the procedure:
Do not eat, drink, smoke or chew gum at least 30 min prior to swab collection. Ensure the swab tip does not come into contact with any surface prior to collection. Put the Stabilizer tube upright to prevent the liquid inside the tube from spilling. Be sure to move the swab over the entire cheek and to moisten it with saliva.

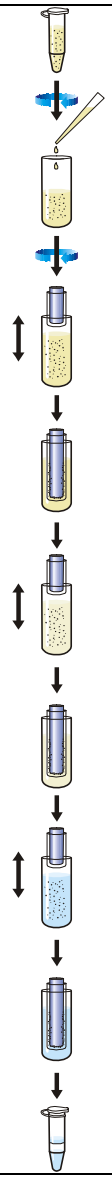
1. 	Open swab package and remove swab without touching the tip.
2. 	Insert swab into the mouth and rub swab tip firmly against the inner cheeks for about 30 seconds on each side.
3. 	Unscrew lid from Stabilizer tube and put it aside for further use. Insert swab into the Stabilizer tube.
4. 	Break swab at breaking point. Discard the broken handle part.
5. 	Screw lid tightly onto the Stabilizer tube again.
6. 	Shake tube for 15 sec to mix buccal cells and DNA Stabilizer reagent.
7. 	Label Stabilizer tube with donor name and collection date using provided label.

Scheme of the InviMag® SalivaGene DNA Kit /KF96

Please read protocols prior the start of the preparation carefully

Important: Prefill all plates with the appropriate buffers and volumes as described below before starting a run.

Tip Plate:	Insert the KF96 Tip Comb for DW magnets on a Tip Plate*
Binding Plate:	Prepare the Binding plate as described below
Washing Plate_1:	Add **500 µl Wash Buffer I** to a 2.0 ml Deep Well Plate
Washing Plate_2:	Add **600 µl Wash Buffer II** to a 2.0 ml Deep Well Plate
Washing Plate_3:	Add **600 µl Wash Buffer II** to a 2.0 ml Deep Well Plate
Elution Plate:	Add **70 µl** or 100 µl*** Elution Buffer** to a KF Elution Plate (same size as Tip Plate)



<u>Extraction of human DNA:</u>
Transfer 500 µl of the stabilized sample into the Lysis Plate, add 20 µl Proteinase S and start the program “InviMag SalivaGene hDNA KFFLEX or InviMag SalivaGene hDNA SWAB KFFLEX”.

After the lysis step, the user will be prompted to add 200 µl **Binding Buffer A** *(follow preparing instructions)* and 20 µl **SNAP Solution** during a pause.

DNA binds to magnetic particles

Magnetic separation

Washing of the particle fixed genomic DNA

Magnetic separation

Elution of nucleic acids

Magnetic Separation

Pure DNA

* Elution Plates and Tip Plates are identically. Use one provided Elution Plate as a Tip Plate.
** 70 µl is the recommended elution volume for Swab DNA extractions (Runfile: InviMag SalivaGene hDNA SWAB KFFLEX)
*** 100 µl is the recommended elution volume for all other Saliva DNA extractions (Runfile: InviMag SalivaGene hDNA KFFLEX)

Protocol 1: Human genomic DNA extraction from stabilized material

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer A*** *– see instruction page: 10*

Important: *For working with the KingFisher instrument, please carefully read the manufacturer's documents !*

1. Switch on the KF96 / KFflex96 instrument
2. Prefill all Deep Well Plates as described below.

Note*: Please avoid evaporation of the prefilled buffer components by sealing the Deep Well Plates with a sealing foil or with parafilm!*

Binding Plate:	Prefill the Binding Plate (2 ml DWP) in each cavity you want to use with 20 µl **Proteinase S**. Carefully transfer 500 µl of the stabilized sample into the cavities of the Plate
Washing plate_1:	Add 500 µl **Wash Buffer I** into the cavities of a 2 ml Deep Well Plate
Washing plate_2:	Add 600 µl **Wash Buffer II** into the cavities of a 2 ml Deep Well Plate
Washing plate_3:	Add 600 µl **Wash Buffer II** into the cavities of a 2 ml Deep Well Plate
Elution Plate:	Add 70** or 100 µl*** **Elution Buffer** into the cavities of the KF Elution Plate

3. Choose the program **"InviMag SalivaGene hDNA SWAB KFFLEX"** or **"InviMag SalivaGene hDNA KFFLEX"** and press the "START" button. Use the "InviMag SalivaGene hDNA SWAB KFFLEX" protocol for samples from swabs, use the "InviMag SalivaGene hDNA KFFLEX" protocol for samples from all other collection devices.
4. Load the prefilled plates into the KingFisher instrument by following the description given on the display and confirm every step by pressing the button "START".

When all prefilled plates are loaded into the KingFisher machine, press the button "START" again to execute the assay file. The run will last approximately 60 minutes.

After protein degradation, the machine will be paused.

Important: *Mix the bottle with the* ***SNAP Solution*** *by vigorously vortexing!*

200 µl **Binding Buffer A** and 20 µl **SNAP Solution** have to be added to each sample containing cavity in the Binding Plate. After addition of both reagents, press the "START" button once again to continue with the run. From here, the machine will finish the purification process without any further user interaction.

** 70 µl is the recommended elution volume for Swab DNA extractions (Runfile: InviMag SalivaGene hDNA SWAB KFFLEX)
*** 100 µl is the recommended elution volume for all other Saliva DNA extractions (Runfile: InviMag SalivaGene hDNA KFFLEX)

Protocol Proposal: Bacterial DNA extraction from stabilized material

Please read the instructions carefully and conduct the prepared procedure.

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1. Switch on the KF96 / KFflex96 instrument

Binding Plate setup:
Prefill each well of the Binding Plate (2 ml DWP) with 20 µl of 10mg/ml Lysozyme
Carefully transfer 500µl of the sample into the cavity of the Binding Plate.
Incubate the plate at 37°C for 10 min, or at RT for 20-30 minutes depending on the cell wall of the bacteria of you interest.
Adaption of Lysozyme amount and incubation times may be necessary.
During incubation time prefill the other Deep Well Plates as follows:

Washing plate_1:	Add 500 µl **Wash Buffer I** into the cavities of a 2 ml Deep Well Plate
Washing plate_2:	Add 600 µl **Wash Buffer II** into the cavities of a 2 ml Deep Well Plate
Washing plate_3:	Add 600 µl **Wash Buffer II** into the cavities of a 2 ml Deep Well Plate
Elution Plate:	Add 70** or 100 µl*** **Elution Buffer** into the cavities of the KF Elution Plate

After this step add 20 µl of Proteinase S and proceed as indicated in Protocol 1, step 3.

Lysozyme is available from Carl Roth, Thermo Fisher and others in different concentrations and package sizes.

For self-programming of the KF96 and KFflex96 instrument

Reagent info

Lysis Plate			Microtiter DW 96 plate	
	Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
	Salivagene Sample	500	-	Reagent
	Proteinase S	20	-	Reagent

Washing Plate 1			Microtiter DW 96 plate	
	Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
	Wash Buffer I	500	-	Reagent

Washing Plate 2			Microtiter DW 96 plate	
	Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
	Wash Buffer II	600	-	Reagent

Washing Plate 3			Microtiter DW 96 plate	
	Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
	Wash Buffer II	600	-	Reagent

Elution Plate			KingFisher 96 KF plate	
	Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
	Elution Buffer	70** 100***	-	Reagent

Tip Plate			Microtiter DW 96 plate	
	Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**

Dispensed reagents

Lysis Plate			Microtiter DW 96 plate	
	Name	**Step**	**Well volume [µl]**	**Total reagent volume [µl]**
	Binding Buffer + SNAP Solution	Adjust Binding Conditions	220	-

** 70 µl Runfile: InviMag SalivaGene hDNA SWAB KFFLEX
*** 100 µl Runfile: InviMag SalivaGene hDNA KFFLEX

Steps data

	Tip	96 DW tip comb	
	Pick-Up	Tip Plate	
	Lysis Step	Lysis Plate	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:20:00, Medium
		Heating temperature [°C]	60
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
	Adjust Binding Conditions	Lysis Plate	
		Message	Add Buffer 200 µl and Beads 20 µl
		Dispensing volume [µl]	220
	Reagent(s)	Name	Binding Buffer + SNAP Solution
		Volume [µl]	220
	Binding Step	Lysis Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	16
	Wash Step 1	Washing Plate 1	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:30, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	7
	Wash Step 2	Washing Plate 2	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	7

	Wash Step 3	Washing Plate 3	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	7
	Drying Step	Washing Plate 3	
		Dry time	00:03:30
		Tip position	Outside well / tube
	Elution Step	Elution Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium
	Mixing / heating:	Mixing time, speed	00:10:00, Slow
		Heating temperature [°C]	60
		Preheat	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	30
	Bead Removal Step	Washing Plate 3	
		Release time, speed	00:00:30, Fast
	Leave	Tip Plate	

Troubleshooting

Problem	Cause	Comments and suggestions
low amount of DNA	Sample not correctly stabilized	Follow the protocol for sample collection & stabilization, pp 12-14
	low percentage alcohol used or buffers are not diluted correctly	repeat purification procedure with a new sample and/or prepare a new bottle of Wash Buffer if a wrong concentration of ethanol was used Recommendation: It is possible to replace the Elution Buffer with water, but then take care about the effect of water for photometric measurement: low pH may reduce measuring values and fake a low DNA yieldOur **Elution Buffer** contains 10 mM Tris-HCl pH 8.5-9, no EDTA.
degraded or sheared DNA		Recommendation: Our Stabilization systems stabilize for up to one year (Swabs 6 months), For longer storage the material may be frozen at -20°C, but here avoid repeated thawing and freezing cycles of the material.
problems with subsequent applications (e.g. in PCR)	ethanol in the eluted DNA	verify in the log if the recommended dry time was reached By heating the samples to 80°C ethanol may be evaporated from the eluates very fast.
	salt in the eluate	**Wash Buffer** should be stored at and used at RT verify **Wash Buffer** on the precipitation of salt if there are precipitations dissolve this by carefully warming up to 30°C
A_{260}/A_{280} ratio for purified DNA is low	Sample not correctly stabilized	Follow the protocol for sample collection & stabilization, pp 12-14
A_{260}/A_{280} ratio for purified DNA delivers strange values	DNA Concentration in sample is too low for the measuring range ≤ 5 ng/µl	Background effect in the photometer check in the description of your photometer

Appendix

KingFisher™ BindIt Software 3.2, 3.3 or higher version 4.0

BindIt software 3.2 or higher versions were and may be used to create assay files for the KFmL, KF96/KFflex96 or KF-Duo instruments. The provided assay file(s) can either be transferred onto the corresponding workstation(s) or be started directly from within the BindIt software after assay import. Please keep in mind, that assay(s) run from within the BindIt software are not stored in the workstation memory.

Important: *Be advised that BindIt SW 3.2 or higher versions use a new unique file extension. Therefore, it is not possible to import assay files created with BindIt 3.2 or higher versions into older BindIt software versions! Please ask your local Thermo Scientific distributor for a software update.*

***Note**:* *When creating assay files for usage with KingFisher™ instruments in combination with Microtiter Deep Well plates (e.g. Thermo Electron), it is essential to use the KingFisher™ software 3.2 or higher versions for assay development because this software version includes the correct adjustments for the microtiter plate. It is highly recommended to use Thermo Microtiter Deep Well plates with KF96 / KFflex96 / KF-Duo workstations to ensure the best purification result.*

Minimum system requirements for BindIt Software 3.2 or higher versions

PC requirements	
Supported operating systems	MS Windows XP Pro with SP3, Windows Vista SP2, Windows 7
Disk space	500 MB free disk space
Processor	Intel Pentium ≥ 1 GHz
Memory	1 GB RAM
USB ports available	1 (for KF96 / KFflex96 / KFDuo connection)
Pointing device	Mouse or equivalent is required
CD-ROM drive	1

If the actual Windows Service Packs are not installed on the corresponding lab computer, they can be downloaded from the Microsoft web pages: http://www.microsoft.com/.

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 compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long-template PCR.

Storage of DNA
A working stock of DNA can be stored at 2-8˚C for several weeks. For long-term storage the DNA should be stored at -20˚C. However, storage at -20°C may cause shearing, particularly if the DNA is exposed to repeated freeze-thawing cycles.
Note that the used elution buffer will affect its stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

Drying, dissolving and pipetting DNA
Avoid over-drying genomic DNA after ethanol precipitation. It is better to air dry than to use a vacuum, although vacuum drying can be used with caution.
Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings can cause shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

DNA yield
The amount of purified DNA depends on sample source, transport conditions, storage, and age of the sample.

Ordering information

Product	Package size	Catalogue No.
InviMag® SalivaGene DNA Kit/ KF96	1 x 96 preps	7435060100
InviMag® SalivaGene DNA Kit/ KF96	5 x 96 preps	7435060200
InviMag® SalivaGene DNA Kit/ KF96 w/o plastic	1 x 96 preps	7435060150
InviMag® SalivaGene DNA Kit/ KF96 w/o plastic	5 x 96 preps	7435060250
SalivaGene Collection Module II	5 container	1035212100
SalivaGene Collection Module II	50 container	1035212200
SalivaGene Collection Module II	125 container	1035212300
SalivaGene Swab Comfort	50 pieces	1035231200
SalivaGene Swab Comfort	300 pieces	1035231300

Related products

PSP® SalivaGene DNA Kit	50 preparations	1035200200
PSP® SalivaGene DNA Kit	250 preparations	1035200300

KingFisher™ 96 and consumables	
KingFisher 96, Magnetic Particle Processor,100-240V,50/60Hz	5400500
KingFisher 96 Head for Deep Well plate	24073430
KingFisher 96 tip comb for PCR magnets, 8 x 10 pcs / box	97002514
KingFisher 96 tip comb for KF magnets, 10 x 10 pcs / box	97002524
KingFisher 96 tip comb for DW magnets 10 x 10 pcs / box	97002534
KingFisher 96 KF plate (200ul) 48 plates / box	97002540
Microtiter deep well 96 plate, 50 plates/box	95040450

Possible suppliers for Isopropanol:

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

Fa. Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

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

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