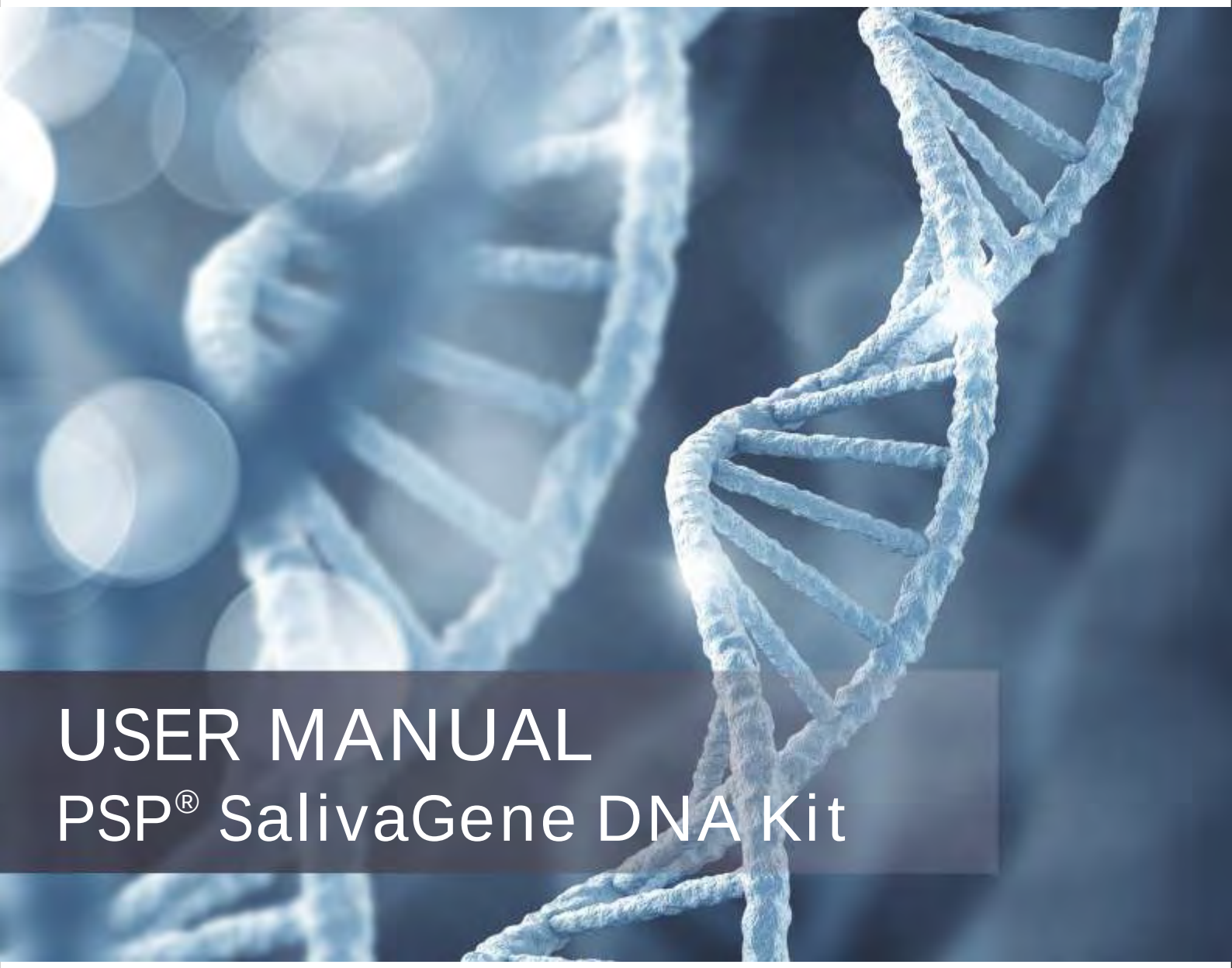
INVITEK
Molecular



USER MANUAL
PSP® SalivaGene DNA Kit

for purification of total DNA from SalivaGene stabilized clinical swab (mouth brushes) and saliva sample

CE IVD

REF 1035200X00

Invitek Molecular GmbH · D-13125 Berlin

Instruction for PSP® SalivaGene DNA Kit

The **PSP® SalivaGene DNA Kit** purifies **SalivaGene** stabilized genomic and bacterial DNA quantitatively from saliva and swabs (e.g. mouth brushes).

The **PSP® SalivaGene DNA Kit** in combination with the **SalivaGene Collection Module II, SalivaGene Collector** or the **SalivaGene Swab Comfort** is a modular system using a stabilization solution for collection, stabilization, storage and transportation of swab or saliva material without degradation of DNA.

The kit has been designed for isolation of genomic, mitochondrial and bacterial DNA for *in vitro* diagnostic analysis.

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

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

Not for *in-vitro* diagnostic use in countries where the EU Directive 98/79/EC on *in vitro* medical devices is not recognized.

Trademarks: Invisorb®, PSP®, MSB®, RTP® 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.

PSP® is a registered trademark of Invitek Molecular GmbH.

© 2020 Invitek Molecular, all rights reserved.

Contents

Kit content of the PSP® SalivaGene DNA Kit

	50 extractions	250 extractions
Catalogue Number	1035200200	1035200300
Lysis Buffer HLT	15 ml	60 ml
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 40 ml)	empty bottle (final volume 200 ml)
Proteinase K	for 1.1 ml working solution	for 5 x 1.1 ml working solution
Wash Buffer HLT	30 ml (final volume 50 ml)	105 ml (final volume 175 ml)
Wash Buffer	2 x 18 ml (final volume 2 x 60 ml)	2 x 60 ml (final volume 2 x 260 ml)
Elution Buffer M	15 ml	30 ml
RTA Spin Filter Set	50	5 x 50
RTA Receiver Tubes	50	5 x 50
1.5 ml Receiver Tubes	50	5 x 50
Manual	1	1
Initial steps	Fill 40 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Dilute **Proteinase K** with 1.1 ml ddH_2O. Mix thoroughly until completely dissolving. Add 20 ml of 96-100% Isopropanol to the bottle **Wash Buffer HLT,** mix thoroughly and store with tightly closed cap. Add 42 ml 96-100% ethanol to each bottle **Wash Buffer**	Fill 200 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Dilute each tube **Proteinase K** with 1.1 ml ddH_2O. Mix thoroughly until completely dissolving. Add 70 ml of 96-100% Isopropanol to the bottle **Wash Buffer HLT,** mix thoroughly and store with tightly closed cap. Add 200 ml 96-100% ethanol to each bottle **Wash Buffer**

Content of SalivaGene Collection Sets

SalivaGene Collection Sets	50 pieces	
SalivaGene Collection Module II	1035212200	
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 **PSP® SalivaGene DNA Kit**, except **dissolved Proteinase K** 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: 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. Therefore, the dissolved Proteinase K is stable as indicated on the kit package.

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

Wash Buffer HLT charged with isopropanol should be appropriately sealed and stored at room temperature.

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

Quality control and product warranty

Invitek Molecular warrants the correct function of the **PSP® SalivaGene DNA Kit** for applications as described in this manual. Purchaser must determine the suitability of the Product for its particular use. Should any Product fail to perform the applications as described in the manual, Invitek Molecular will check the lot and if Invitek Molecular investigates a problem in the lot, Invitek Molecular will replace the Product free of charge.
Invitek Molecular reserves the right to change, alter, or modify any Product to enhance its performance and design at any time.
In accordance with Invitek Molecular's EN ISO 13485 certified Quality Management System the performance of all components of the **PSP® SalivaGene DNA Kit** 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 **PSP® SalivaGene DNA Kit** or other Invitek Molecular products, please do not hesitate to contact us. A copy of Invitek Molecular's terms and conditions can be obtained upon request or are presented at the Invitek Molecular webpage www.invitek-molecular.com.

For technical support or further information please contact:

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

Intended use of the PSP® SalivaGene DNA Kit

The **PSP® SalivaGene DNA Kit** is suitable for reproducible purification of total DNA from 500 µl stabilization media of swab and saliva samples in forensic, human-identity, genetic, biosecurity and pathogen and for *in vitro* diagnostic 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 four modules, three collection sets and one extraction module, useable independently or in combination. Also included is an easy to handle protocol for DNA purification by precipitation.

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 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.

The kit complies with EU Directive 98/79/EC on in vitro medical devices. However, it is not for in-vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Product use limitation

The kit is neither validated for the isolation of genomic DNA from serum, plasma, fungi nor from parasites or 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 Invitek Molecular Product for any particular use. Invitek Molecular does not provide for validation of performance characteristics of the Product with respect to specific applications.

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

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

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

The chemicals and the 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 with its contents is 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.invitek-molecular.com for each Invitek Molecular Product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

Invitek Molecular has not tested the liquid waste generated by the **PSP® SalivaGene DNA Kit** 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 according to local safety regulations.

European Community risk and safety phrases for the components of the **PSP® SalivaGene DNA Kit** and for the **SalivaGene Stabilization Sets** to which they apply are listed below as follows:.

Proteinase K

Danger

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

Saliva DNA Stabilizer

Warning

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

Lysis Buffer HLT

Warning

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

H302: Harmful if swallowed.
H315: Causes skin irritation.
H317: May cause an allergic skin reaction.
H318: Causes serious eye damage.
H319: Causes serious eye irritation.
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

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

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

Product characteristic of the PSP® SalivaGene DNA Kit

Starting material	Yield	Time	Ratio
500 µl of stabilization buffer (SalivaGene DNA Stabilizer) for **SalivaGene Collector/ SalivaGene Collection Module II**	up to 10 µg (depends of kind of starting material)	30 min	A_{260} : A_{280} 1.7 – 2.0
500 µl of stabilization buffer (SalivaGene DNA Stabilizer) for **Swab/ SalivaGene Swab Comfort**	up to 1 µg (depends on the donor)		

The **PSP® SalivaGene DNA Kit** uses the cost efficient **PSP® Technology** (Preanalytical-Sample-Processing). It is a modular designed system for collection, stabilization, storage and transportation of saliva or swab samples completed with DNA purification. The system combines the use of prefilled **SalivaGene Collection Sets** for saliva or swab sample collection with the storage and stabilization of respective specimen. The DNA is protected during transportation. Bacterial samples are pre-lysed, bacterial growth is inhibited. The procedure is completed by a very efficient and fast isolation method (up to 500 µl, about 30 min) which results in high quality total DNA. This kit has been designed for isolation of DNA from host organism, as well as for DNA from pathogenic bacteria and eukaryotes for *in vitro* diagnostic analysis.

After sample collection the saliva or swab sample it is transferred (as described), in one of the available **SalivaGene Collection Sets**, prefilled with **SalivaGene DNA Stabilizer**. The raw material is lysed under specific conditions. The **SalivaGene DNA Stabilizer** effects inactivation of DNases and prevents degradation of DNA, it preserves the microorganism titre and pre-lyses bacteria.

The sample may be stored under this buffer at -20° for a several years. For the DNA extraction an aliquot may be used, the residual sample is stored for further extractions.

500 µl aliquots of the sample are used in the DNA isolation procedure. The proteins are degraded with Proteinase K, followed by a binding of the DNA to a Spin Filter while contaminants pass this filter. The DNA is eluted in Elution Buffer M provided with the kit.

Yields are from sample to sample depending on factors as the nature of the sample (swab or saliva) and the DNA contents of the respective samples.

The Kit procedure requires neither phenol/ chloroform extraction nor alcohol precipitation. A minimal interaction by the user allows safe handling of potentially infectious samples. The whole process 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 Analysis*, RFLP / AFLP*- Analysis
- Restriction Enzyme Digestion
- HLA-Typing
- Pathogen Detection

Sampling and sample storage

Starting material

The amount of starting material used in PSP® SalivaGene DNA Kit procedures can vary slightly, 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.

Swab:

The DNA of swab samples (collected using **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.

Invitek 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.

Principle and procedure

The **PSP® SalivaGene DNA Kit** procedure comprises following steps:

- collection of the material using one of the **SalivaGene Collection Sets**
- lysis of cells
- protein digestion
- binding genomic DNA to the membrane of a RTA Spin Filter
- washing the membrane and elimination of contaminants and ethanol
- elution of pure genomic DNA

This manual contains 2 protocols, according to the different requirements of the starting materials.

Protein digestion
Proteins will be digested in the lysed samples in the presence of **Proteinase K**.

Binding genomic DNA
By adding **Binding Solution (Isopropanol)** to the lysate, optimal binding conditions will be adjusted.
Each lysate is then applied to an RTA® Spin Filter and genomic DNA is adsorbed to the membrane.

Removing residual contaminations
Contaminants are efficiently washed away using **Wash Buffer HLT** and **Wash Buffer**, while the total DNA remains bound to the membrane.

Elution of pure genomic DNA
Genomic DNA is eluted from the Spin Filter using 50 - 100 µl **Elution Buffer M**. The eluted DNA is ready for use in different downstream applications.

Yield and quality of DNA

The amount of purified DNA in the **PSP® SalivaGene DNA Kit** procedure from different samples depends on the sample type and sample source. Yield and quality of isolated DNA is suitable for any molecular-diagnostic detection system. The diagnostic tests should be performed according to manufacturer's specifications.

Important points before starting a protocol

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

- Always change pipette tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipette tips.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Discard contaminated gloves.
- 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, bring all reagents to room temperature. Where necessary, gently mix and re-dissolve any precipitates by incubation at 30°C. Swirl gently to avoid foaming.

1. Adjust the Thermomixer to 56°C.
2. Warm up the needed amount of **Elution Buffer M** to 56°C
3. Label the needed amount of RTA Spin Filter (lid).
4. Label the needed amount of 1.5 ml Receiver Tubes (per sample: 1 Receiver Tube).
5. Add the needed µl ddH2O to reaction tube with Proteinase K (see below). Vortex for 10 s
6. Fill the needed volume of Isopropanol into the empty bottle labeled with Binding Solution
7. Add the needed amount of Ethanol to the **Wash Buffer**. Mix for 5 s
8. Add the needed amount of Isopropanol to the **Wash Buffer HLT**. Mix for 5 s

50 DNA extractions:
Fill 40 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Dilute **Proteinase K** with 1.1 ml ddH2O. Mix thoroughly until completely dissolving.
Add 20 ml of 96-100% Isopropanol to the bottle **Wash Buffer HLT**, mix thoroughly and store with tightly closed cap.
Add 42 ml 96-100% ethanol to each bottle **Wash Buffer**

250 DNA extractions:
Fill 200 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Dilute each tube **Proteinase K** with 1.1 ml ddH2O. Mix thoroughly until completely dissolving.
Add 70 ml of 96-100% Isopropanol to the bottle **Wash Buffer HLT**, mix thoroughly and store with tightly closed cap.
Add 200 ml 96-100% ethanol to each bottle **Wash Buffer**

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.invitek-molecular.com under each Invitek Molecular kit and kit component.

- Microcentrifuge
- Thermomixer (for 56°C)
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipette and pipette tips
- Vortexer
- Reaction tubes (1.5 ml or 2.0 ml)
- dd H_2O
- 96 - 100 % Ethanol
- Isopropanol *

*The **PSP® SalivaGene DNA Kit** 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
Ordering No. 6752

Applichem
2-Propanol für die Molekularbiologie
Ordering No. A3928

Sigma
2-Propanol
Ordering No. 59304-1L-F

Important indications

1. Process only as many samples as the microcentrifuge allows to process.
2. Sample and buffers should be thoroughly mixed and should have room temperature
3. The elution can be done by using lower amount of **Elution Buffer M**. This may result in a higher concentration of DNA. However, pay attention that minimum volume for elution is 30 µl, this will reduce the yield. Elution volume between 2 x 50 µl up to 200 µl will realize comparable results regarding yield.
4. **Elution Buffer M** does not contain EDTA.

PSP®-Treatment with SalivaGene Collector

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

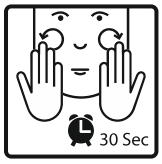
Limitations of the procedure:
Do not eat, drink, smoke or chew gum at least 30 min prior to saliva collection. Collecting 2 ml of saliva may require several minutes. Putting some grains of sugar on the tongue helps to produce saliva and does not interfere with the stabilization.

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 PSP® SalivaGene DNA Kit

Please read protocols prior the start of the preparation carefully

---

Transfer 500 µl of the stabilized sample from the **SalivaGene Collection Sets** to a 2 ml Safe Lock Tube

EXTRA STEP FOR BACTERIAL DNA

Add 10 µl of **Lysozyme** (10 mg/ ml)* and incubate for 10 min at 37°C under continuously shaking or every 5 minute flicking the tube.

1. Protein removal

Add 20 µl of **Proteinase K** and **200 µl Lysis Buffer HLT**, vortex 15 seconds.
Incubate for 10 minutes at 56°C under continuously shaking (1200 RPM if applicable).

(Take an aliquot of Elution Buffer M and preheat to 56°C)

2. Realizing optimal binding conditions
Add 700 µl of **Binding Solution.**
vortex 15 seconds.

3. Binding of the DNA
Transfer the 700 µl of the mixture onto a RTA Spin Filter Set.
Wait 1 min.
Centrifuge for 2 minutes at 11.000 x g (or 11.000 rpm).
Discard the filtrate.
Place the RTA Spin Filter back into the 2.0 ml RTA Receiver Tube. Transfer the rest of the mixture onto the RTA Spin Filter and close the RTA Spin Filter.
Centrifuge for 1 min with 11.000 x g (11.000 rpm).
Discard the filtrate.

4. Washing of the Spin Filter
Place the Spin Filter into a new RTA Receiver Tube and add 600 µl **Wash Buffer HLT** to the Filter. Centrifuge at 11.000 x g (11.000 rpm) for 1 minute. Discard filtrate.

Add 700 µl **Wash Buffer** to the Filter. Centrifuge at 11.000 x g (11.000 rpm) for 1 minute. Discard filtrate.

Repeat the second washing step. Discard filtrate.

Remove ethanol by centrifugation for 4 minutes at maximum speed.

5. Elution of DNA
Place the RTA Spin Filter into a 1.5 ml Receiver Tube and add 50 – 100 µl of preheated **Elution Buffer M (56°C)**. Incubate for 1 minute at room temperature. Close the Spin Filter and centrifuge for 1 minute at 11.000 x g (11.000 rpm).

Protocol I: Human DNA extraction from stabilized material by the PSP® SalivaGene DNA Kit

Please read the instructions carefully and conduct the prepared procedure.

Important *Transfer the needed amount of **Elution Buffer M** into a Receiver Tube (not included in the kit) and place the tube at 56°C.*

1. Protein removal

Transfer 500 µl of **stabilized saliva sample** from the **SalivaGene Collection Sets** into a 2 ml Safe Lock Tube

Add 20 µl **Proteinase K** and 200 µl **Lysis Buffer HLT**, mix thoroughly by pulse-vortexing for 15 sec. after each addition.

Incubate for 10 min at 56°C while continuously shaking (1200RPM).

2. Binding of DNA to the RTA Spin Filter

Add 700 µl **Binding Solution** and mix thoroughly by pulse-vortexing for 15 sec.

Briefly centrifuge the 2.0 ml Safe Lock Tube to remove drops from the inside of the lid.

Take a RTA Spin Filter Set. Transfer 700 µl of the mixture onto the RTA Spin Filter. Close the RTA Spin Filter and incubate for 1 min at RT.

Centrifuge for 2 min with 11.000 x g (11.000 rpm). Discard the filtrate. Place the RTA Spin Filter back into the 2.0 ml RTA Receiver Tube. Transfer the rest of the mixture onto the RTA Spin Filter and close the RTA Spin Filter. Centrifuge for 1 min with 11.000 x g (11.000 rpm). Discard the filtrate.

3. First washing of the RTA Spin Filter

Place the RTA Spin Filter into a new 2 ml RTA Receiver Tube.

Add 600 µl **Wash Buffer HLT** to the RTA Spin Filter. Close the RTA Spin Filter. Centrifuge for 1 min with 11.000 x g (11.000 rpm). Discard the filtrate and place the RTA Spin Filter into a new 2.0 ml RTA Receiver Tube.

4. Second washing of the RTA Spin Filter

Add 700 µl **Wash Buffer** to the RTA Spin Filter and close the RTA Spin Filter. Centrifuge for 1 min with 11.000 x g (11.000 rpm). Discard the filtrate and place the RTA Spin Filter into a new 2.0 ml RTA Receiver Tube.

Repeat the washing step and discard the filtrate.

Place the RTA Spin Filter back into the 2.0 ml RTA Receiver Tube. Centrifuge for 4 min at maximum speed (14.000 rpm) to eliminate the ethanol.

5. Elution of DNA

Place the RTA Spin Filter into a 1.5 ml Receiver Tube. Add 50 –100 µl of preheated (56°C) **Elution Buffer M** and close the RTA Spin Filter. Incubate at RT for 1 min.

Centrifuge for 1 min with 11.000 x g (11.000 rpm).

Discard the RTA Spin Filter and store the eluted DNA at 4 °C.

Note: *The DNA can also be eluted with a lower volume of **Elution Buffer M**. It is also possible to repeat the elution step with equal volume of **Elution Buffer M**. This will lead to increased total yield. However, minimum volume for the elution is 30 µl.*

Note: *The centrifugation steps were made with the **Centrifuge 5415 D from Eppendorf.** The indicated **rpm** are referring to this centrifuge.*

Protocol II: Bacterial DNA extraction from stabilized material by the PSP® SalivaGene DNA Kit

Please read the instructions carefully and conduct the prepared procedure.

Important *Transfer the needed amount of **Elution Buffer M** into a Receiver Tube (not included in the kit) and place the tube at 56°C.*

Important: ***Bacterial swabs must be taken by medical staff.***

1. Protein removal

Transfer 500 µl of **stabilized saliva sample** from the **SalivaGene Collection Sets** into a 2.0 ml Safe Lock tube.

Add 10 µl of **Lysozyme** (10 mg/ ml)* and incubate for 10 min at 37°C under continuously shaking or every 5 minute flicking the tube.

Add 20 µl of **Proteinase K** and 200 µl **Lysis Buffer HLT** mix thoroughly by pulse-vortexing for 15 sec. after each addition. Incubate for 10 min at 56°C under continuously shaking (1200 RPM) or every 5 minutes flicking the tube.

Incubate the sample for additional 10 minutes under continuously shaking at 95°C. This step will lead to a thermic disintegration of bacterial cell wall structures. Human Genomic DNA of the cellular material might be broken by this step! After Lysis Step spin down shortly.

2. Binding of DNA to the RTA Spin Filter

Add 700 µl **Binding Solution** and mix thoroughly by pulse-vortexing for 15 sec.

Briefly centrifuge the 2.0 ml Safe Lock Tube to remove drops from the inside of the lid.

Take a RTA Spin Filter Set. Transfer 700 µl of the mixture onto the RTA Spin Filter. Close the RTA Spin Filter and incubate for 1 min at RT.

Centrifuge for 2 min with 11.000 x g (11.000 rpm). Discard the filtrate. Place the RTA Spin Filter back into the 2.0 ml RTA Receiver Tube. Transfer the rest of the mixture onto the RTA Spin Filter and close the RTA Spin Filter. Centrifuge for 1 min with 11.000 x g (11.000 rpm). Discard the filtrate.

3. First washing of the RTA Spin Filter

Place the RTA Spin Filter into a new 2 ml RTA Receiver Tube.

Add 600 µl **Wash Buffer HLT** to the RTA Spin Filter. Close the RTA Spin Filter. Centrifuge for 1 min with 11.000 x g (11.000 rpm). Discard the filtrate and place the RTA Spin Filter into a new 2.0 ml RTA Receiver Tube.

4. Second washing of the RTA Spin Filter

Add 700 µl **Wash Buffer** to the RTA Spin Filter and close the RTA Spin Filter. Centrifuge for 1 min with 11.000 x g (11.000 rpm). Discard the filtrate and place the RTA Spin Filter into a new 2.0 ml RTA Receiver Tube.

Repeat the washing step and discard the filtrate.

Place the RTA Spin Filter back into the 2.0 ml RTA Receiver Tube. Centrifuge for 4 min at maximum speed (14.000 rpm) to eliminate the ethanol.

5. Elution of DNA

Place the RTA Spin Filter into a 1.5 ml Receiver Tube. Add 50 –100 µl of preheated (56°C) **Elution Buffer M** and close the RTA Spin Filter. Incubate at RT for 1 min.

Centrifuge for 1 min with 11.000 x g (11.000 rpm).

Discard the RTA Spin Filter and store the eluted DNA at 4 °C.

Note: *The DNA can also be eluted with a lower volume of **Elution Buffer M**. It is also possible to repeat the elution step with equal volume of **Elution Buffer M**. This will lead to increased total yield. However, minimum volume for the elution is 30 µl.*

***for gram-positive bacteria, not included in the kit*

Troubleshooting

Problem	Cause	Comments and suggestions
low amount of DNA	insufficient cell lysis	increase lysis time , reduce amount of starting material overloading of Spin Filter reduces yield
	insufficient cell lysis due to decreased **Proteinase K** activity	repeat the DNA purification procedure with a new sample and freshly prepared **Proteinase K** stock solution. Be sure to store the stock solution at –20°C.
	insufficient lysis time	the DNA purification procedure should be done not earlier than 1 hour after sampling
	inefficient binding of DNA to the membrane e.g. due to insufficient mixing with **Binding Solution**	overloading RTA Spin Filter reduces yield use correct amount of **Binding Solution** mix sample **with Binding Solution** by pipetting up and down 4-5 times or by vortexing (5 sec) prior to transfer the sample onto filter
	low percentage alcohol used	repeat purification procedure with a new sample
	incomplete elution	increase incubation time with preheated **Elution Buffer M** to 5 - 10 min , elute twice with each 100 µl **Elution Buffer M** use higher volume of Elution Buffer M.
	low sample DNA-concentration	elute the DNA with lower volume of **Elution Buffer M**
	pH of water incorrect (acidic)	low pH may reduce DNA yield. Ensure that the pH of the water is at least 7.0 or use **Elution Buffer M (**contains only 10 mM Tris – HCL, no EDTA)
degraded or sheared DNA	old material with several freeze thaw cycles	avoid repeated thawing and freezing of the material
clogged Spin Filter	insufficient lysis	perform isolation as described in protocols
	too much starting material	increase lysis time with lysis buffer **SalivaGene DNA Stabilizer** increase centrifugation time and/or speed reduce amount of starting material
problems with subsequent applications (e.g. in PCR)	ethanol in the eluted DNA	verify if the recommended centrifugation time was reached increase centrifugation time for the elimination of ethanol if necessary
	salt in the eluate	**Wash Buffer** should be stored and used at RT verify **Wash Buffer** on the precipitation of salt. If there are precipitations dissolve this by careful warming up to 30°C
	reduced sensitivity of amplification reaction	adjust the volume of eluate added as template in the amplification reaction
A_{260}/A_{280} ratio for purified DNA is low	insufficient cell lysis and protein degradation due to decreased **Proteinase K** activity	see above under “ low amount of DNA”
	insufficient lysis due to insufficient **Proteinase K** activity	see above under “ low amount of DNA”

Appendix

General notes on handling DNA

Starting material
This kit is designed for extraction of DNA from saliva and swabs. These materials show big variation in DNA contents. The purification of some apoptotic DNA is normal.

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, and long-template PCR.

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

Note that the solution in which the nucleic acid is eluted in 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 let it 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 causes 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.

Ordering information

Product	Package Size	Catalogue No.
PSP® SalivaGene DNA Kit	50 preparations	1035200200
PSP® SalivaGene DNA Kit	250 preparations	1035200300
SalivaGene Swab Comfort	50 pieces	1035231200
SalivaGene Swab Comfort	300 pieces	1035231300
SalivaGene Collector	50 pieces	1035211200
SalivaGene Collection Module II	50 container	1035212200

***Possible suppliers for Isopropanol:**

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

Applichem
2-Propanol für die Molekularbiologie
Ordering No. A3928

Sigma
2-Propanol
Ordering No. 59304-1L-F

INVITEK
Molecular

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

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

www.invitek-molecular.com

0061035200 V-01-2020