

User manual Invisorb[®] Spin Swab Kit

For genomic DNA purification from from clinical swab material



REF 1035120x00



STRATEC Molecular GmbH, D-13125 Berlin



Instruction for Invisorb® Spin Swab Kit

The **Invisorb® Spin Swab Kit** is the ideal tool using the Invisorb® technologies for manual isolation and purification of DNA from swabs like buccal, nasal, pharyngeal and vaginal swabs for *in vitro* diagnostic analysis.

The kit is neither validated for the isolation of DNA from stool samples, blood samples, fungi, plants, or viruses, nor for purification of RNA.



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

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

Trademarks: Invisorb®. 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 6,110,363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

Invisorb® is a registered trademark 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.

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Kit contents of Invisorb® Spin Swab Kit

Store diluted Proteinase K at – 20 °C, but repeated freezing and thawing will reduced the activity dramatically. Dividing the Proteinase K into aliquots and storage at – 20°C is recommended.

	5 DNA preps	50 DNA preps	250 DNA preps
Catalogue No.	1035120100	1035120200	1035120300
Lysis Buffer G	2 x 2 ml	50 ml	160 ml
Binding Buffer A	2 x 1 ml (ready to use)	9 ml (final volume 30 ml)	36 ml (final volume 120 ml)
Proteinase K	for 250 µl working solution	for 1.1 ml working solution	for 5 x 1.1 ml working solution
Wash Buffer	15 ml (ready to use)	2 x 18 ml (final volume 2 x 60 ml)	2 x 60 ml (final volume 2 x 200 ml)
Elution Buffer	2 ml	15 ml	30 ml
Spin Filter	5	50	5 x 50
2.0 ml Receiver Tubes	5	50	5 x 50
1.5 ml Receiver Tubes	5	50	5 x 50
Sterile swabs	5	50	5 x 50
Manual	1	1	1
Initial steps	<p>The Wash Buffer is ready to use</p> <p>The Binding Buffer A is ready to use</p> <p>Add 250 µl dd H₂O to the tube with Proteinase K, mix thoroughly until completely dissolving and store at - 20°C!</p> <p>Incubate the needed amount of Elution Buffer at 65°C</p>	<p>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.</p> <p>Add 42 ml of 96-100 % Ethanol to the bottle Wash Buffer, mix thoroughly and keep the bottle always firmly closed !</p> <p>Add 1.1 ml dd H₂O to the tube with Proteinase K, mix thoroughly until completely dissolving and store at - 20°C!</p> <p>Incubate the needed amount of Elution Buffer at 65°C</p>	<p>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.</p> <p>Add 140 ml of 96-100 % Ethanol to each bottle Wash Buffer , mix thoroughly and keep the bottle always firmly closed</p> <p>Add 1.1 ml dd H₂O to each tube with Proteinase K, mix thoroughly until completely dissolving and store at - 20°C!</p> <p>Incubate the needed amount of Elution Buffer at 65°C</p>

Symbols

	Lot number
	Catalogue number
	Expiry date
	Consult operating instructions
	Temperature limitation
	Do not reuse
	Manufacturer

Storage

All buffers and kit contents of the **Invisorb® Spin Swab Kit** except dissolved Proteinase K should be stored at room temperature (RT) and are stable for at least 12 months under these conditions. Dissolved Proteinase K must be stored at – 20°C.

Wash Buffer charged with ethanol should be stored at room temperature and should be appropriately sealed. If there are any precipitates within the provided solutions dissolve these precipitates by carefully warming up to room temperature.

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

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **Invisorb® Spin Swab 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, 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 ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **Invisorb® Spin Swab 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 **Invisorb® Spin Swab Kit** 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 **Invisorb® Spin Swab Mini Kit** is the ideal tools, using the Invisorb® technology for manual isolation and purification of genomic DNA from f swabs like buccal, nasal, pharyngeal and vaginal swabs for *in vitro* diagnostic analysis.

For reproducible and high yields appropriate sample storage is essential. The purified DNA can be used for *in vitro* diagnostic analysis only.

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 is in compliance with EU Directive 98/79/EC on in vitro medical devices. But it is not for in-vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Product use limitation

The kit is validated neither for the isolation of DNA from stool samples, blood, tissue, fungi or viruses, nor for isolation and purification of 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 conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All Products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 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 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.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 **Invisorb® Spin Swab 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.

Patient specimens must always be considered as potentially infectious. Samples from risk patients must always be labeled and handled under suitable safety conditions. Observe all federal, state and local safety and environmental regulations.

Observe the usual precautions for nucleic acid applications. It is essential that all reagents and materials used for DNA isolation are free from DNases.

European Community risk and safety phrases for the components of the **Invisorb® Spin Swab Kit** to which they apply, are listed below as follows:

Proteinase K



danger

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

H319:	Causes serious eye irritation.
H315:	Causes skin irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
P305+P351+P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.

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 Invisorb® Spin Swab Kit

Starting material	Yield	Time for preparation	Ratio
buccal, nasal, pharyngeal, vaginal swabs	up to 6 µg; depends of kind of starting material	30 min	$A_{260} : A_{280}$ 1.7 – 2.0

The **Invisorb® Spin Swab Kit** uses the well-established Invisorb® technology to provide an extremely fast way to isolate genomic DNA from above named starting material. The purified DNA is free of contaminants and enzyme inhibitors and performs reliably in downstream applications such as PCR. Purifications require no phenol or chloroform extraction or alcohol precipitation. No toxic or hazardous chemicals like chaotropic components are used.

The kit is designed for simultaneous processing of multiple samples. The procedure requires minimal interaction by the user, allowing safe handling of potentially infectious samples. The procedures are designed to avoid sample-to-sample cross-contamination.

Purified DNA is eluted in a low-salt buffer (without EDTA) or water. 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*
- RFLP-Analysis
- Restriction Enzyme Digestion
- HLA Typing
- Cloning*

For further information please contact: Phone: + 49 (0) 30 9489 2901, 2910 in Germany and from foreign countries phone: + 49 (0) 30 9489 2907

Principle and procedure

The **Invisorb® Spin Swab Kits** simple procedure comprises following steps:

1. lysis of cells
2. binding the genomic DNA to the membrane of a Spin Filter
3. washing the membrane and elimination of ethanol
4. elution of genomic DNA

Sample collection and storage:

To collect a sample, scrape the swab firmly against inside of each cheek 6 times. Air-dry the swab for at least 2 h after collection or use them fresh prepared. Ensure that the person providing the sample has not consumed any food or drink in the 30 min prior to sample collection. Best results are obtained if the swab stays in the lysis solution during lysis procedure. Use of poor quality starting material influences yield of purified DNA. This protocol is recommended for every common swab, like e.g. the following swab types: C:E:P: (Omni Swab from Whatman), cotton swab, Superswabs, Copan-Swab or DRACON tip from Hardwood Products company, CellProjects or Hain Diagnostika)

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.

Lysis with Proteinase K

Samples are lysed under anti-chaotropic conditions at elevated temperature and continuously shaking. Lysis is performed in the presence of **Lysis Buffer G** and **Proteinase K**. By crushing or grinding the sample under liquid nitrogen, the lysis efficiency is dramatically increased and lysis time is reduced. Using rodent tails an overnight lysis is possible. Unlysed sample parts should be removed before the binding step.

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

Binding genomic DNA

By adding **Binding Buffers A** to the lysate, optimal binding conditions are adjusted. Each lysate is then applied to an Invisorb® Spin Filter and genomic DNA is adsorbed onto the membrane as the lysate is drawn through by centrifugal force as contaminants pass through.

Removing residual contaminants

Remaining contaminants and enzyme inhibitors are efficiently removed in two efficient wash steps using **Wash Buffer**, while the genomic DNA remains bound to the membrane.

Elution of pure genomic DNA

Genomic DNA “ready for use” is eluted from the Spin Filter using **50 - 100 µl Elution Buffer** or water. Invisorb purified DNA has $A_{260} : A_{280}$ ratios of 1.6-2.0 and absorbance scans show a symmetric peak at 260 nm confirming purify.

Eluting twice by splitting the elution volume in two parts leads to little increase of DNA yield.

The usage small elution volumes may raise DNA concentration.

Elution volumes should be at least 50 µl or 150 µl. The eluted DNA is ready for use in different downstream applications.

Important notes

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 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, 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.
- All centrifugation steps are carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Discard gloves if they become contaminated.
- Do not combine components of different kits, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by trained personnel.

Preparing reagents and buffers for the Invisorb® Spin Swab Kit

Before starting a run, bring all reagents to room temperature. Where necessary, gently mix and re-dissolve any precipitates by incubation at 30°C. Swirl gently to avoid foaming.

Lysis Buffer G, Binding Buffer A and **Elution Buffer** are ready to use.

Add the needed volume of ddH₂O (see Kit Contents page 3) to the reaction tube with **Proteinase K**. Vortex for 5 sec, store diluted **Proteinase K** at -20°C.

1. adjust the thermomixer to 65°C
2. warm up the needed amount of **Elution Buffer** to 65°C (50 - 100 µl **Elution Buffer** are needed per sample)
3. label the needed amount of 2.0 ml Receiver Tubes
4. place Spin Filters into labeled 2.0 ml Receiver Tubes
5. label the needed amount of 1.5 ml Receiver Tubes
6. add the needed ddH₂O (see Kit Contents page 3) to the reaction tube with **Proteinase K**, vortex for 5 sec, store dissolved **Proteinase K** at -20°
7. add the needed amount of ethanol to the **Wash Buffer**

5 DNA-extractions:

Add **250 µl dd H₂O** to the tube **Proteinase K**, mix thoroughly until completely dissolving and store at -20°C!

The **Wash Buffer** and the **Binding Buffer A** is ready to use

50 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 **1.1 ml dd H₂O** to the tube **Proteinase K**, mix thoroughly until completely dissolving and store at -20°C!

Add **42 ml of 96 - 100% ethanol** to the bottle **Wash Buffer**, mix thoroughly and always keep the bottle firmly closed!

250 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 **1.1 ml dd H₂O** to each tube **Proteinase K**, mix thoroughly until completely dissolving and store at -20°C!

Add **140 ml of 96 - 100% ethanol** to the bottle **Wash Buffer**, mix thoroughly and always keep the bottle firmly closed!

Reagents and equipment to be supplied by user

- Microcentrifuge
- Thermomixer (for 65°C)
- Measuring cylinder (250 ml)
- Pipette and pipette tips
- Disposable gloves
- Reaction tubes (1.5 ml or 2.0 ml)
- dd H₂O
- Vortexer
- 96-100 % Ethanol
- Isopropanol

*The **Invisorb® Spin Swab 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
Order no. 6752

Applichem

2-Propanol für die Molekularbiologie
Order no. A3928


Sigma

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

Important indications

1. Invisorb® Spin Filter can also purify low amounts of RNA besides DNA. For the elimination of RNA (if necessary) add 40 µl **RNase A** (10 mg/ml) before adding the **Binding Buffer A**. After that vortex shortly and incubate the sample at room temperature for 5 minutes. Then go on as described in the protocol.
2. The elution can be done by using lower amount of **Elution Buffer** (min 50 µl). This may result in a higher DNA-concentration.
3. Eluting twice with each with 50 µl **Elution Buffer** is also possible and produces slightly higher yield of DNA.
4. **Co-purification of RNA:** The kit co-purifies DNA and RNA when both are present in the same sample. Samples which contain high level of RNA, RNA will be co-purified. RNA may inhibit some downstream enzymatic reactions, although it does not affect PCR. If RNA-free genomic DNA is required. **RNase A** should be added to the sample before addition of **Binding Buffer A**, to digest the RNA.

Scheme of the Invisorb® Spin Swab Kit

 <p>Genomic DNA</p>	<p>Please read protocols prior the start of the preparation</p> <p>Pipette 600 µl Lysis Buffer G and 20 µl Proteinase K to the sample 5 – 10 s mix thoroughly (e.g. vortexing) Incubate at 65°C while continuously shaking for 15 min</p> <p>Add 300 µl Binding Buffer A (<i>follow preparing instructions</i>), mix thoroughly Place a Spin Filter into a 2.0 ml Receiver Tube, Transfer the suspension onto the Spin Filter. Centrifugation for 2 min at 11.000 x g (11.000 rpm). Discard filtrate</p> <p>Add 700 µl Wash Buffer onto Spin Filter, Centrifuge for 1 min at 11.000 x g (11.000 rpm) Discard filtrate, Place the Spin Filter again into the 2.0 ml Receiver Tube Repeat the Washing step Discard filtrate, Place Spin Filter again into the 2.0 ml Receiver Tube Centrifuge for 4 min at maximum speed for ethanol removal</p> <p>Place the Spin Filter into a 1.5ml Receiver Tube, Add 50 - 100 µl prewarmed Elution Buffer Incubate at room temperature for 3 min Centrifuge for 1 min at 11.000 x g (11.000 rpm) Discard the Spin Filter</p> <p>The eluate contains “ready to use” DNA”</p>
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Protocol 1: DNA Extraction from swab 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: 9

Important: Prewarm the Elution Buffer to 65°C (e.g. transfer the needed volume into a reaction tube and place the tube at the appropriate temperature)

1. Lysis of the starting material

Transfer 600 µl of **Lysis Buffer G** and 20 µl of **Proteinase K** into a 1.5 ml Reaction Tube. Transfer the swab into the so prepared tube and incubate the sample at 65°C for 15 minutes under continuously shaking (e.g. by using a thermo mixer)

Important: To get maximum yield of DNA it is essential to leave the swab during the complete lysis time into the reaction tube. It is possible to cut the shaft of the swab, so that you can close the cap of the reaction tube. It is also possible to do the lysis step with opened cap. The removing of the swab from the reaction tube ahead of time will lead to a dramatically reduced final yield!
After lysis time carefully squeeze out the swab on the wall of the tube and discard the swab.

2. Realizing optimal binding conditions

Add 300 µl of **Binding Buffer A** to the reaction tube and mix thoroughly. Set a Spin Filter into a 2.0 ml Receiver Tube and transfer the suspension onto the Spin Filter. Centrifugation for 2 min at 11.000 x g (11.000 rpm). Open the Receiver Tube, take the Spin Filter from the Receiver Tube and discard the filtrate.

3. Washing of the Spin Filter

Place the Spin Filter back into the 2.0 ml Receiver Tube and add 700 µl **Wash Buffer** to the Filter. Centrifugation at 11.000 x g (11.000 rpm) for 1 min. Take the Filter from the Receiver Tube, discard the filtrate and then place the Spin Filter again into the Receiver Tube. Repeat the washing step once. Discard the filtrate again and put the Spin Filter back into the Receiver Tube. Close the Tube and centrifuge for 4 min at maximum speed (for complete removal of Ethanol).

4. Elution of DNA

Place the Spin Filter into a new 1.5 ml Receiver Tube and add 50 –100 µl of prewarmed **Elution Buffer**. Incubation for 1 min at room temperature. Close the Receiver Tubes and centrifuge for 1 min at 11.000 x g (11.000 rpm). Discard the Spin Filter. The filtrate contains the pure DNA.

Note: The DNA can also be eluted with a lower volume of Elution Buffer. It is also possible to do the elution step two times with equal volumes of Elution Buffer. These will lead to slightly increased total yield. But pay attention, that minimum volume for the elution is 40 µl.

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

Protocol 2: DNA Extraction from swab material especially for isolation of bacterial DNA (particular gram+ bacteria or other hard to lyse pathogens)

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: 9

Important: Prewarm the Elution Buffer to 65°C (e.g. transfer the needed volume into a reaction tube and place the tube at the appropriate temperature)

1. Lysis of the starting material

Transfer 600 µl of **Lysis Buffer G** and 20 µl of **Proteinase K** into a 1.5 ml Reaction Tube. Transfer the swab into the so prepared tube and incubate the sample at 65°C for 15 minutes under continuously shaking (e.g. by using a thermomixer).

Important: To get maximum yield of DNA it is essential to leave the swab during the complete lysis time into the reaction tube. It is possible to cut the shaft of the swab, so that you can close the cap of the reaction tube. It is also possible to do the lysis step with opened cap. The removing of the swab from the reaction tube ahead of time will lead to a dramatically reduced final yield!
After lysis time carefully squeeze out the swab on the wall of the tube and discard the swab.

Incubate the sample for additional 30 minutes under continuously shaking at 95°C. This step will lead to a thermic disintegration of bacterial cell wall structures.

2. Realizing optimal binding conditions

Add 300 µl of **Binding Buffer A** to the reaction tube and mix thoroughly. Set a Spin Filter into a 2.0 ml Receiver Tube and transfer the suspension onto the Spin Filter. Centrifugation for 2 min at 11.000 x g (11.000 rpm).

Open the Receiver Tube, take the Spin Filter from the Receiver Tube and discard the filtrate.

3. Washing of the Spin Filter

Place the Spin Filter back into the 2.0 ml Receiver Tube and add 700 µl **Wash Buffer** to the Filter. Centrifugation at 11.000 x g (11.000 rpm) for 1 min. Take the Filter from the Receiver Tube, discard the filtrate and then place the Spin Filter again into the Receiver Tube. Repeat the washing step once. Discard the filtrate again and put the Spin Filter back into the Receiver Tube. Close the Tube and centrifuge for 4 min at maximum speed (for complete removal of Ethanol).

4. Elution of DNA

Place the Spin Filter into a new 1.5 ml Receiver Tube and add 50 –100 µl of prewarmed **Elution Buffer**. Incubation for 1 min at room temperature. Close the Receiver Tubes and centrifuge for 1 min at 11.000 x g (11.000 rpm).

Discard the Spin Filter. The filtrate contains the pure DNA.

Note: The DNA can also be eluted with a lower volume of Elution Buffer. It is also possible to do the elution step two times with equal volumes of Elution Buffer. These will lead to slightly increased total yield. But pay attention, that minimum volume for the elution is 40 µl.

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

Troubleshooting

Problem	Cause	Comments and suggestions
clogged Spin-Filter	insufficient lysis and/ or too much starting material	incubate the swab during the complete lysis time into the reaction tube. increase lysis time. increase centrifugation speed. reduce amount of starting material.
low concentration of extracted DNA	too much Elution Buffer	elute the DNA with lower volume of Elution Buffer .
Degraded or sheared DNA	old material	old material often contains degraded DNA.
low amount of extracted DNA	insufficient lysis incomplete elution insufficient mixing with Binding Buffer A	increase lysis time. reduce amount of starting material. overloading of spin filter reduces yield! prolong the incubation time with Elution Buffer to 5-10 min or repeat elution step once again. take higher volume of Elution Buffer mix sample with Binding Buffer A by pipetting or by vortexing prior to transfer the sample onto the spin column.

Appendix

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 – 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 it's 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.

DNA yield

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

Ordering information

Product	Package Size	Catalogue No.
Invisorb® Spin Swab Kit	5 preps	1035120100
Invisorb® Spin Swab Kit	50 preps	1035120200
Invisorb® Spin Swab Kit	250 preps	1035120300

Single components for the Invisorb® Spin Swab Kit

Lysis Buffer G	60 ml	1035121100
Binding Buffer A (add 21 ml)	15 ml	1035122100
Wash Buffer (add 42 ml)	18 ml	1035123200
Elution Buffer	15 ml	1035124000

Related products

PSP® SalivaGene DNA Kit	50 preparations	1035200200
PSP® SalivaGene DNA Kit	250 preparations	1035200300
SalivaGene® Collection Module II	5 container	1035210600
SalivaGene® Collection Module II	50 container	1035210700
SalivaGene® Collector	5 pieces	1035210100
SalivaGene® Collector	50 pieces	1035210200
SalivaGene® Buccal Swab	5 pieces	1035230100
SalivaGene® Buccal Swab	50 pieces	1035230200

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



strat**ec**●●
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