



USER MANUAL

Invisorb[®] Spin Tissue Mini Kit

for purification of genomic DNA from 0.5 - 40 mg tissue sample; mouse tail;
10 – 10⁶ eucaryotic cells; swabs, as well as from food samples of animal origin

Instruction for Invisorb® Spin Tissue Mini Kit

The **Invisorb® Spin Tissue Mini Kit** is the ideal tool using the Invisorb® technologies for manual isolation and purification of DNA from small amounts of various human and animal tissues (e.g. muscle, liver, heart and brain), biopsy material, rodent tail, eukaryotic cell pellets and swabs. The kit is neither for isolation of DNA from stool samples, blood samples, bacteria, fungi, plants, or viruses, nor for purification of RNA.



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.

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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 Invitek Molecular GmbH.

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 Tissue Mini Kit

	250 DNA extractions
Catalogue No.	1032100300
Lysis Buffer G	120 ml
Binding Buffer A	2 x 9 ml (final volume 2 x 30 ml)
Proteinase S	6 x 2 ml
Wash Buffer	2 x 45 ml (final volume 2 x 150 ml)
Elution Buffer	120 ml
Spin Filter	5 x 50
2.0 ml Receiver Tubes	5 x 50
1.5 ml Receiver Tubes	5 x 50
Manual	1
Initial steps	<p>Add 21 ml 99.7% Isopropanol to each Binding Buffer A. Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.</p> <p>Add 105 ml of 96-100% Ethanol to the bottle Wash Buffer, mix thoroughly and always keep the bottle firmly closed!</p>

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 **Invisorb® Spin Tissue Mini Kit** 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 Buffer charged with Ethanol should be appropriately sealed and stored at room temperature.

Binding Buffer A charged with Isopropanol should be appropriately sealed and stored at room temperature.

Quality control and product warranty

Invitek Molecular warrants the correct function of the Invisorb® Spin Tissue Mini 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 **Invisorb® Spin Tissue Mini 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 Tissue Mini 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:

Email: techsupport@invitek-molecular.com or contact your local distributor.

Intended use

The **Invisorb® Spin Tissue Mini Kit** is the ideal tool, using the Invisorb® technology for manual isolation and purification of genomic DNA from fresh or frozen human and animal tissues, biopsy material, rodent tail and insects, as well as animal origin food samples and eukaryotic cells, and cell pellets. Furthermore, the **Invisorb® Spin Tissue Mini Kit** allows the isolation of apoptotic DNA ladders from cells.

Apoptosis is a form of cell death and characterized morphologically by cell shrinkage, blebbing of the membrane and compaction of nuclear chromatin and cytoplasm. Furthermore, apoptosis is associated with the activation of Ca^{2+} and Mg^{2+} depending endonucleases resulting in the double-strand cleavage of the chromosomal DNA at the internucleosomal sites. The cleavage of genomic DNA leads to fragmentation of the DNA into multiple fragments of 180bp multimeric bands. A characteristic apoptosis DNA ladder is obtained if the apoptotic DNA is resolved by a classical agarose gel electrophoresis.

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

Product use limitation

The kit is validated neither for the isolation of DNA from stool samples, blood, bacteria, 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 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 Invisorb® Spin Tissue Mini 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 regulation. Patient specimens must always be considered as potentially infectious. Samples from risk patients must always be labelled 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 Tissue Mini Kits** to which they apply are listed below as follows:

Proteinase S



Danger

H317-H318-P280-P305+P351+P338

Lysis Buffer G



Warning

H319-H412-P280-P305+P351+P338

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.

P280: Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

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

outside of USA: 1 – 352 – 323 – 3500

in USA : 1 – 800 – 535 – 5053

Product characteristic of the Invisorb® Spin Tissue Mini Kit

Starting material	Yield	Time for preparation	Ratio
0.5 – 40 mg tissue sample up to 1.0 cm mouse tail up to 0.5 cm rat tail biopsy material insects	up to 50 µg depends on type and amount of tissue samples 0.2 - 1.2 µg per mg tissue/ 10 - 40 µg per rodent tail	15 min after lysis	$A_{260} : A_{280}$ 1.6 – 2.1
10 – 10 ⁶ eukaryotic cells swabs	max. 6 µg from human cells max. 10 µg from animal cells		

The **Invisorb® Spin Tissue Mini Kit** use the well-established Invisorb® technology to provide an extremely fast way to isolate genomic DNA from above named starting material and amount as well as apoptotic DNA from cells. The purified DNA is free of contaminants and enzyme inhibitors and performs reliably in downstream applications such as PCR. Purifications requires 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*
- Restriction Enzyme Digestion
- HLA Typing
- Sequencing
- Southern Blot

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

Principle and procedure

The Invisorb® Spin Tissue Mini Kit 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

This manual contains 7 protocols, according to the different requirements of the starting materials

Sample collection and storage:

Tissue sample / biopsy material / frozen section:

Best results are obtained with fresh material or material that has been immediately frozen and stored at – 20°C or – 80°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size. Use of poor quality starting material influences yield of purified DNA. The amount of purified DNA in the Invisorb® Spin Tissue Mini Kit procedure using 0.5-40 mg tissue sample, depends on kind of starting material. The thawing process could proceed, directly in **Lyse Buffer G**.

Rodent tail:

Best results are obtained with fresh material or material that has been immediately frozen and stored at – 20°C or – 80°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size. A long-time lysing also leads to degradation of DNA. Crushing the rodent tails reduces lysis time.

Insects:

Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C. Insects especially with chitin mail must be homogenized before lysis (for example by grinding with mortar and pestle under liquid nitrogen). The sample can be stored for a short time at 2-8°C in **Lysis Buffer G**.

Cells grown in suspension:

Spin up to 1×10^8 cells for 5 min at 300 x g (1.500 rpm*). Discard supernatant and remove all media completely, taking care not to disturb the cell pellet. At this point cells may be frozen (at -20°C or - 80°C) for future use or may be used immediately.

Cells grown in a monolayer:

Aspirate the media completely from the cells and continue immediately with the lysis step. Alternatively cells can be detached by trypsination (cultivation in larger culture vessels, like dishes > Ø 35 mm, flasks > 12.5 cm²). Transfer cells to a 50 ml reaction tube, pellet by centrifugation at 300 x g (1.500 rpm*) for 5 min and aspirate supernatant completely.

Buccal swabs:

To collect a sample, scrape the swab firmly against inside of each cheek 6 times. Air-dry the swab for at least 2h 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)

CSF and Bone marrow on haematological slides:

Best results are obtained with fresh material: But commonly the sample will be dried. They have to be stored cooled at 4°C in a dried surrounding.

Note: After Proteinase S digestion, tissue samples can be stored in Lysis Buffer G for up to 6 months at -20°C without any reduction of DNA quality

Lysis with Proteinase S

Samples are lysed under non-chaotropic conditions at elevated temperature and continuously shaking. Lysis is performed in the presence of **Lysis Buffer G** and **Proteinase S**.

Proteinase S is colored blue, this is for tracking the correct addition of the small volume enzyme. 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.

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 - 200 µl** or **300 – 500 µl Elution Buffer** or water. Invisorb purified DNA has $A_{260}:A_{280}$ ratios of 1.6-2.1 and absorbance scans show a symmetric peak at 260 nm confirming purity.

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

The usage of small elution volumes may raise DNA concentration.

Elution volumes should be at least 50 µl or 200 µl. The eluted DNA is ready for use in different downstream applications.

Scheme of the Invisorb® Spin Tissue Mini Kit

 <p>Genomic DNA</p>	<p>Please read protocols prior the start of the preparation</p> <p>Tissuecrush sample into little pieces or grind with liquid nitrogen and transfer to a 1.5 ml tube (not provided)</p> <p>pipette 400 µl Lysis Buffer G and 40 µl Proteinase S to the sample 5 – 10 s mix thoroughly (e.g. vortex) incubate at 52°C under constant shaking until lysis is completed (overnight lysis is also possible)</p> <p>centrifuge for 2 min at 11.000 x g (11.000 rpm)</p> <p>transfer supernatant into a new 1.5 ml tube (not provided)</p> <p>add 200 µl Binding Buffer A (<i>follow preparing instructions</i>) and vortex.</p> <p>place a Spin Filter into a 2.0 ml Receiver Tube transfer lysate onto the Spin Filter</p> <p>incubate at room temperature for 1 min</p> <p>centrifuge for 3 min at 13.500 x g (12.000 rpm) and discard filtrate</p> <p>add 550 µl Wash Buffer onto Spin Filter and centrifuge for 1 min at 11.000 x g (11.000 rpm)</p> <p>discard filtrate,</p> <p>place the Spin Filter again into the 2.0 ml Receiver Tube repeat the Washing step</p> <p>discard filtrate</p> <p>place Spin Filter again into the 2.0 ml Receiver Tube and centrifuge for 4 min at maximum speed for Ethanol removal</p> <p>place the Spin Filter into a 1.5 ml Receiver Tube. add 50 - 200 µl prewarmed Elution Buffer incubate at room temperature for 3 min centrifuge for 1 min at 11.000 x g (11.000 rpm) and discard the Spin Filter</p> <p>the eluate contains “ready to use” DNA*</p>
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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 must notify Invitex Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the Invitex 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.
- 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

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 and **Elution Buffer** are ready to use.

1. adjust the thermomixer to 52°C
2. warm up the needed amount of **Elution Buffer** to 52°C
(50 - 200 µl **Elution Buffer** are needed per sample)
3. label the needed amount of 2.0 ml Receiver Tubes (per sample: 1 Receiver Tube)
4. place Spin Filters into labeled 2.0 ml Receiver Tubes
5. label the needed amount of 1.5 ml Receiver Tubes (per sample: 1 Receiver Tube)
6. add the needed amount of Isopropanol to the **Binding Buffer A**
7. add the needed amount of Ethanol to the **Wash Buffer**

250 DNA extractions

Add 21 ml 99.7% Isopropanol to each **Binding Buffer A**. Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.

Add 105 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 52°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
- optional: RNase A (10 mg/ml)
- Isopropanol*

*The **Invisorb® Spin Tissue Mini 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. From liver sample use only up to 20 mg tissue, excessive amount of tissue can result in inefficient lysis. If information is not available on the DNA content of the sample, we recommend using less than 40 mg tissue. 25 mg tissue will provide an average yield of 5 to 40 µg genomic DNA.
2. Incomplete removal of the cell culture media will dilute the lysate and affect lysis.
3. The kit procedure is also suitable for purifying DNA from very small amounts of starting material. If the sample has less than 5 ng DNA (>1.000 copies), 3-5 µg Carrier (a homopolymer such as poly-dA, poly-dT or gDNA) should be added to the starting material. Ensure that the Carrier DNA does not interfere with the downstream application. In order to prevent any interference of the carrier with the downstream application, a RNA carrier can be used. This can be removed later by RNase digestion.
4. Optimal disruption of tissue is important for obtaining maximum yield and purity of genomic DNA. The most common method of cell disruption is by grinding with a mortar and pestle. With this method, it is possible to reduce the incubation time with the **Proteinase S**. You can also use a rotor stator homogenizer to disrupt the freshly extracted tissue samples. Since the sample is homogenized in **Lysis Solution**, this will ensure satisfactory homogenization.
5. 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.
6. The elution can be done by using lower amount of **Elution Buffer** (min 50 µl). This may result in a higher DNA-concentration.
7. Eluting twice with each with 100 µl **Elution Buffer** is also possible and produces slightly higher yield of DNA.

Protocol 1: DNA isolation from 0.5 - 40 mg tissue, biopsy sample frozen section, insects, rodent tail (up to 1.2 cm) and food sample from animal origin

Please read the instructions carefully and carry out preparatory arrangements in advance.

Attention: *Please be aware, that you must prepare the **Binding Buffer A and the Wash Buffer** – see instruction page: 11*

Prewarm the needed amount of Elution Buffer up to 52°C.

Attention: *When using liver tissue please take not more than 20 mg!*

1. Transfer the starting material into a 1.5 ml reaction tube (not provided). (A mechanical disruption or a cutting of the material will increase the lysis efficiency)
2. Add 400 µl **Lysis Buffer G** and 40 µl **Proteinase S** and vortex thoroughly.
3. Incubate the reaction tube at 52°C until the lysis is complete under constant shaking. For material that is difficult to lyse we recommend to vortex the tube several times.

Optional: *For mouse tail, difficult to lyse material overnight lysis is possible. This step will shear the DNA a little bit, but it will not disturb any downstream reaction.*

4. Centrifuge for 2 min at 11.000 x g (11.000 rpm) to spin down non-lysed material. Transfer the supernatant into a new 1.5 ml tube (not provided).

Optional: *To remove RNA from the sample (if necessary) add 40 µl of **RNase A** (10 mg/ml), vortex shortly and incubate for 5 min at RT*

5. Add 200 µl **Binding Buffer A** and vortex for 10 sec.
6. Place a Spin Filter into a 2.0 ml Receiver Tube. Transfer the suspension onto the Spin Filter and incubate for 1 min. Close Spin Filter and centrifuge at 13.500 x g (12.000 rpm) for 3 min.
7. Discard the filtrate and place the Spin Filter again into the Receiver Tube.
8. Add 550 µl **Wash Buffer**, close Spin Filter and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate, place the Spin Filter again into the Receiver Tube.
9. Repeat the washing step once. Discard the filtrate, put the Spin Filter back into the Receiver Tube and remove the residual Ethanol by final centrifugation for 4 min at maximum speed Place the Spin Filter into a 1.5 ml Receiver Tube and add 200 µl of the prewarmed **Elution Buffer**. Incubate for 3 min at room temperature. Centrifuge for 1 min at 11.000 x g (11.000 rpm)

Note: *The DNA can also be eluted with a lower or a higher volume of Elution Buffer (depends on the expected yield of genomic DNA). However, please note that minimum volume for the elution is 50 µl. If a large amount of DNA is expected, the volume of elution can be increased.*

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

Protocol 2: DNA isolation from 10 – 10⁶ eukaryotic cells/ cell pellets

Please read the instructions carefully and carry out preparatory arrangements in advance.

Attention: Please be aware, that you must prepare the **Binding Buffer A and the Wash Buffer** – see instruction page: 11

Prewarm the needed amount of Elution Buffer up to 52°C.

1. Add 400 µl **Lysis Buffer G** and 40 µl **Proteinase S** to the washed cell pellet and vortex thoroughly. Transfer the complete mixture to a 1.5 ml reaction tube (not provided).
2. Incubate the reaction tube at 52°C until the lysis is complete under constant shaking. For difficult to lyse material we recommend to vortex the tube several times.

Proceed as described in protocol 1 steps 4 – 9.

Protocol 3: Isolation of apoptotic DNA from 10 – 10⁶ eukaryotic cells / cell pellets

Please read the instructions carefully and carry out preparatory arrangements in advance.

Attention: Please be aware, that you must prepare the **Binding Buffer A and the Wash Buffer** – see instruction page: 11

Prewarm the needed amount of Elution Buffer up to 52°C.

1. Add 400 µl **Lysis Buffer G** and 40 µl **Proteinase S** to the washed cell pellet and vortex thoroughly. Transfer the complete mixture to a 1.5 ml reaction tube (not provided).
2. Incubate the reaction tube 15 min at 52°C until the lysis is complete under constant shaking. For difficult to lyse material we recommend to vortex the tube several times.

Proceed as described in protocol 1 steps 4 – 9.

Note: *The kit contains no DNA control!*

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

Protocol 4: DNA isolation from swab or rinsed liquid

Please read the instructions carefully and carry out preparatory arrangements in advance.

Attention: Please be aware, that you must prepare the **Binding Buffer A and the Wash Buffer** – see instruction page: 11

Important Note: For this protocol, you need additional **Lysis Buffer G and Binding Buffer A**.

Prewarm the needed amount of Elution Buffer up to 52 °C.

1. Lysis of the starting material: swab

Transfer 600 µl of **Lysis Buffer G** and 40 µl of **Proteinase S** into a 1.5 ml Reaction Tube. Transfer the swab into the prepared tube and incubate the sample at 52°C for 15 minutes under constant 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 removal of the swab from the reaction tube before complete lysis will lead to a dramatically reduced final yield!

Important: If the swab is in a transport media, spin down the cells at maximum speed for 1 min from the transport media and discard the supernatant. Resuspend the cell pellet with the Lysis Buffer G and add Proteinase S. To get maximum yield of DNA it is essential swab into the reaction tube during the complete lysis time.

After lysis, carefully squeeze out the swab on the wall of the tube and discard the swab.

Note: The cells should not be lysed, because the DNA will be in the transport media and will be lost during this process (for this case contact the technical support).

2. Realizing optimal binding conditions

Add 300 µl of **Binding Buffer A** to the reaction tube and mix thoroughly. Place a Spin Filter into a 2.0 ml Receiver Tube. Transfer the suspension onto the Spin Filter and incubate for 1 min at RT. Close Spin Filter and centrifuge at 13.500 x g (12.000 rpm) for 3 min. Discard the filtrate and place the Spin Filter again into the Receiver Tube. **Proceed as described in protocol 1 step 8.**

1. Lysis of the starting material: rinsed Lysis Buffer

Rinse each swab with 600 µl **Lysis Buffer G**, remove the swab after carefully squeezing and transfer each sample in a 1.5 ml Reaction Tube (provided with the kit). Add 40 µl of **Proteinase S** to the liquid.

Incubate the sample at 52°C for 15 minutes under constant shaking (e.g. by using a thermomixer).

2. Realizing optimal binding conditions

Add 300 µl of **Binding Buffer A** to the reaction tube and mix thoroughly. Place a Spin Filter into a 2.0 ml Receiver Tube. Transfer the suspension onto the Spin Filter and incubate for 1 min at RT. Close Spin Filter and centrifuge at 13.500 x g (12.000 rpm) for 3 min. Discard the filtrate and place the Spin Filter again into the Receiver Tube.

Proceed as described in protocol 1 step 8.

Troubleshooting

Problem	Cause	Comments and suggestions
low yield	storage of starting material	DNA yield is dependent on the type, size, age and storage of starting material. lower yields will be obtained from material that has been inappropriately stored (see page 10)
	insufficient lysis too much starting material	increase lysis time reduce amount of starting material overloading of Spin Filter reduces yield
	insufficient mixing with Binding Buffer A	mix sample with Binding Buffer A by pipetting or by vortexing prior to transfer the sample onto the Spin Filter
	insufficient Ethanol elimination	increase centrifugation time
	incomplete elution	incubate with Elution Buffer for 5-10 min or repeat elution step once again take higher volume of Elution Buffer
	Wash Buffer prepared incorrectly Water used instead of Elution Buffer	make sure that Ethanol has been added to the Wash Buffer before use (see page 4 or 21) the low pH of deionized water from some water purifiers may reduce DNA yield. When eluting with water, ensure that the pH of the water is at least 7.0
clogged Spin-Filter	insufficient lysis	cut tissue into smaller pieces to facilitate lysis, during and after lysis vortex sample vigorously, this will not damage or reduce size of the DNA increase lysis time increase centrifugation speed after lysis spin lysate to pellet unlysed material and continue with the protocol using the supernatant if a substantial gelatinous pellet remains after incubation at 52°C increase amount of Proteinase S to 60 µl and prolong lysis time
	too much starting material	reduce amount of starting material
degraded or sheared DNA	incorrect storage of starting material	ensure that the starting material is stored as described at page 10 avoid thawing of the material
	old material	old material often contains degraded DNA

Problem	Cause	Comments and suggestions
A₂₆₀:A₂₈₀ ratio of purified DNA is low	water used instead of Elution Buffer to measure A ₂₆₀ /A ₂₈₀ inefficient cell lysis	use 10 mM TrisHCl, pH 7.5 instead of water to dilute the sample before measuring purity see "low yield" above
A₂₆₀ : A₂₈₀ ratio of purified DNA is high	high level of residual RNA	perform optional RNase treatment in the protocol
RNA-Contaminations of extracted DNA	high level of residual RNA	perform optional RNase treatment in the protocol
DNA does not perform well in downstream applications	salt carryover Ethanol carryover too much DNA used	ensure that the Wash Buffer has been used at RT ensure the Wash Buffer has added in the correct order ensure that, when washing with Wash Buffer the column is centrifuged for 3 min at 13.000 x g or for 20 min at 3.000 x g Remove the spin column carefully so that the column does not come into contact with the flow through for PCR applications, a single-copy gene can typically be detected after 35 PCR cycles with 100 ng template DNA
DNA sheared	sample repeatedly frozen and thawed sample too old	avoid repeated freezing and thawing of starting material old samples often yield only degraded DNA

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 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.

Handling of DNA

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 tissue or rodent tail sample depends on sample source, transport conditions, storage and age of the sample.

Ordering information

Product	Package Size	Catalogue No.
Invisorb® Spin Tissue Mini Kit	250 preparations	1032100300

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

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0061032100 V-02-2020