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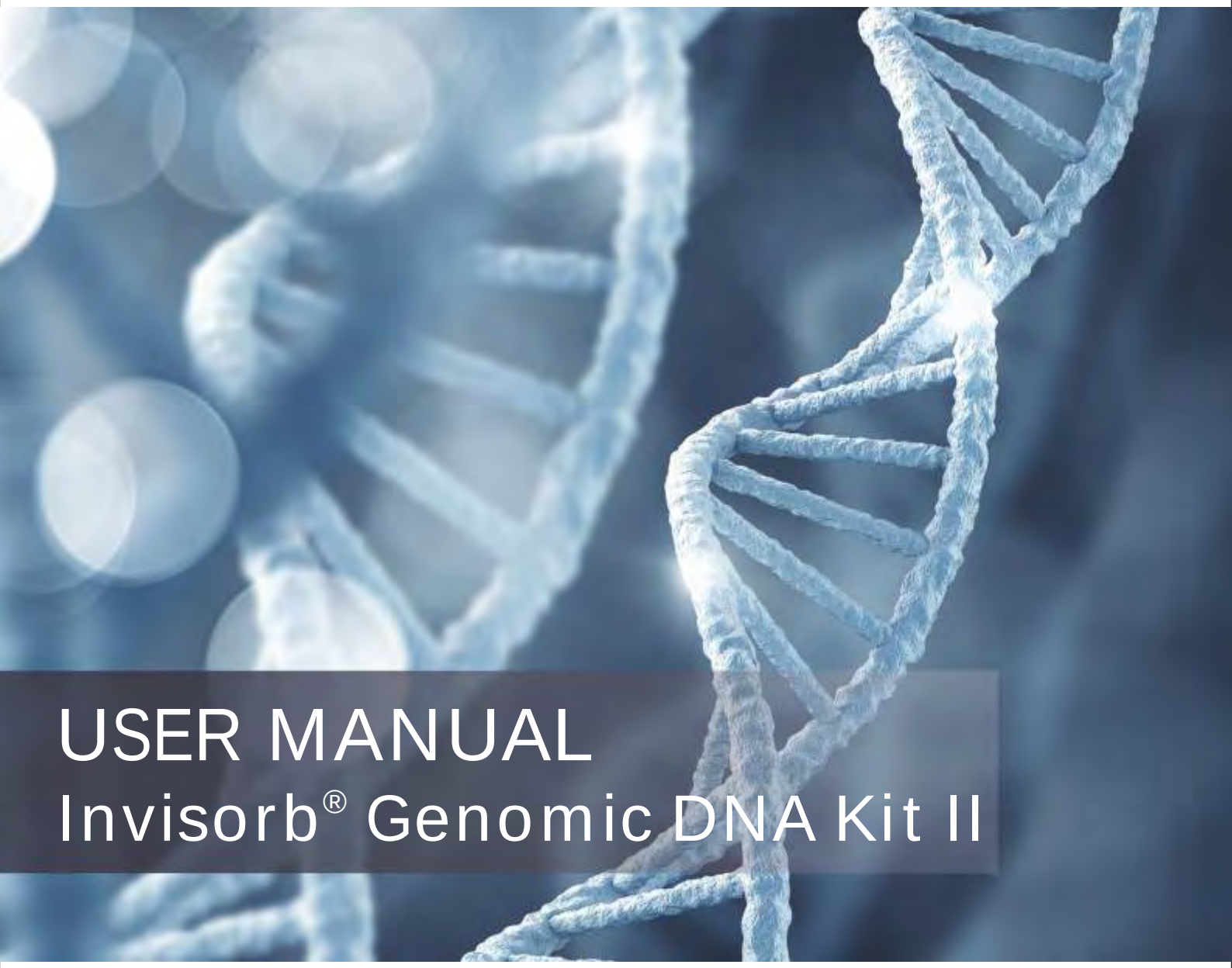

USER MANUAL
Invisorb® Genomic DNA Kit II

For genomic DNA purification from 0.5 - 40 mg tissue sample or mouse tail (batch system)

REF 1032120400

Invitek Molecular GmbH · D-13125 Berlin

Instructions of Invisorb® Genomic DNA Kit II

The **Invisorb® Genomic DNA Kit II** has been developed for a fast and simple isolation of genomic DNA from tissue samples and mouse tail. The kit completely avoids extractions with harmful and toxic organic solvents as well as ethanol precipitations and provides a set of convenient protocols for simultaneous analysis of multiple DNA samples per day
In contrast to column-based systems, the **Invisorb® Genomic DNA Kit II** batch system makes it possible to prepare genomic DNA even from smallest amounts of starting material and requires whether large amounts of starting material nor multiple phenol/ chloroform extractions. Genomic DNA from large numbers of samples can be prepared very fast and with high recovery rates. No special equipment is required.

The **Invisorb® Genomic DNA Kit II** combines the cell and protein destructive properties of chaotropic compounds, which result in the inactivation of endogenous DNases, with binding of cellular DNA to silica particles. Unique physical properties cause the excellent DNA binding capacity of the silica particles.
The excellent quality of the genomic DNA isolated with this kit allows stable long-term storage at 4°C or –20°C.

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

The kit is neither validated for the isolation of viral DNA or the purification of total RNA.

Contents

Kit contents of Invisorb® Genomic DNA Kit II

	500 reactions
Catalogue No.	1032120400
Lysis Buffer G	160 ml
Binding Buffer G	5 x 60 ml
Proteinase K	for 5 x 2 ml working solution
Wash Buffer	4 x 15 ml (final volume 300 ml)
Elution Buffer	200 ml
Manual	1
Initial steps	Add 75 ml distilled water and 210 ml of 96-100% Ethanol to each **Wash Buffer Concentrate**, then mix thoroughly. add 2.0 ml ddH_2O to each **Proteinase K,** mix thoroughly until completely dissolving. Set a water bath, or a Thermal Cycler or a Thermomixer to 50°C (Lysis Buffer G) / 60°C (Elution Buffer)

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® Genomic DNA Kit II,** 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.

Quality control and product warranty

Invitek Molecular warrants the correct function of the **Invisorb® Genomic DNA Kit II** 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® Genomic DNA Kit II** 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® Genomic DNA Kit II** 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

The **Invisorb® Genomic DNA Kit II** is the ideal tool 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.

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

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.

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® Genomic DNA Kit II** 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 **Invisorb® Genomic DNA Kit II** to which they apply, are listed below as follows:

Lysis Buffer G

Warning

H319, H412, P280, P305+P351+P338

Proteinase K

Danger

H315-319-334-335 P280-P305-P351-P338

Binding Buffer G

Danger

H225, H319, H336, P210, P233, P305+351+338

H225: Highly flammable liquid and vapour.
H315: Causes skin irritation.H319: Causes serious eye irritation.
H319: Causes serious eye irritation.
H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335: May cause respiratory irritation.
H336: May cause drowsiness or dizziness.
H412: Harmful to aquatic life with long lasting effects.
P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P233: Keep container tightly closed.
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

Reagents and equipment to be supplied by user

- Microcentrifuge
- Water bath set to 60°C (Thermomixer or Thermal cycler)
- Vortexer
- Microcentrifuge tubes (1.5 ml or 2.0 ml)
- Distilled Water (Milli Q 18M-quality)
- Ethanol (96 -100%)
- PBS Buffer
- TE-Buffer

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 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 **Lyse Buffer G**.

Cells grown in suspension:
Spin up to 1 x 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 > ∅ 35 mm, flasks > 12.5 cm^2). 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. The have to be stored cooled at 4°C in a dried surrounding.

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

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.

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 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 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

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.
Add the needed volume of ddH_2O to the reaction tube with **Proteinase K**. Vortex for 5 sec.

500 DNA extractions
Add 75 ml distilled water and 210 ml of 96-100% Ethanol to each Wash Buffer Concentrate, then mix thoroughly. Add 2.0 ml ddH2O to each Proteinase K, mix thoroughly until completely dissolving! Set a water bath, or a Thermal Cycler or a Thermomixer to 50°C (Lysis Buffer G) / 60°C (Elution Buffer)

Protocol I: DNA isolation from fresh or frozen tissue samples (0.5-40mg)

Please read the instructions carefully and conduct the prepared procedure.

Note: *Incubate Elution Buffer at 60°C. Incubate Lysis Buffer at 50°C for 5 min*

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

1. Place approximately 0.5 – 40 mg of fresh or frozen tissue material into a 1.5 or 2.0 ml reaction tube.

Note: *For tissue material, which is difficult to be lysed (for instance muscle or lung material) we advise grinding the sample under liquid nitrogen. A mechanical grinding of tissue with a glass rod can increase lysis efficiency too.*

2. Add 200 µl **Lysis Buffer G** and 20 µl **Proteinase K** for tissue samples up to 10 mg or 300 µl **Lysis Buffer G** and 20 µl **Proteinase K** for tissue samples >10 mg**.** Incubation of the tube at 60°C (continuous shaking for instance with a Thermomixer increases lysis process) for lysis of starting material.
3. Centrifuge for 1 min at 12 - 14.000 rpm for pelleting particles, which are not lysed. Transfer the supernatant into a new reaction tube (1.5 or 2.0 ml)

Note: ***The Binding Buffer G has to be vortexed thoroughly before use.***

4. Addition of **Binding Buffer G** as described below. Vortex the sample. Incubation for 3 min at room temperature. If the amount of tissue is 0.5 – 10 mg add 400 µl Binding Buffer, if amount is >10 mg then add 600 µl
5. Centrifuge for 30 sec at 10.000 rpm (short spin) to pellet down the silica particles; discard the supernatant carefully.
6. Add 1 ml **Wash Buffer**, then vortex briefly until the pellet is completely resuspended; then centrifuge at 10.000 rpm for 30 sec (short spin). Discard the supernatant carefully.
7. Repeat the step 6 once again, then briefly spin down the residual fluid and remove the residual Wash Buffer as completely as possible by pipetting. Then incubate the open tube in a Vacuum dessicator or a water bath at 60°C for some minutes to completely evaporate the residual ethanol from the Wash Buffer (Thermomixer or Thermal cycler can also be used)
8. Addition of **Elution Buffer** prewarmed to 60°C (Amount concerning to the table below) or of Tris buffered ddH_2O (pH 8.5 - 9.0). Thoroughly resuspend the pellet by pipetting up and down, by manual vortexing. Incubate at 60°C for 3 min.
9. If the amount of tissue is 0.5-5 mg add 150 µl Elution Buffer, if it is 5-10mg add 200µl Elution Buffer and from 10 – 40 mg add 300 – 400 µl Elution Buffer.
10. Centrifuge at 14.000 rpm for 1 min and transfer the DNA containing supernatant into a new reaction tube. Be very careful while pipetting the supernatant to avoid carry-over of silica particles (leave at least 2 µl supernatant above the pellet).

Note: *Regardless of how carefully the final DNA solution was separated from the Silica pellet, some residual particles always remain in the solution, which may affect the activity of DNA-modifying enzymes. To avoid associated problems, we recommend centrifugation of the DNA solution for 2 minutes at 14.000 rpm, each time prior to use. A DNA aliquot for further use should be taken immediately after centrifugation from the top of the solution.*

Protocol II: Isolation of DNA from mouse tail

Please read the instructions carefully and conduct the prepared procedure.

Note: *Incubate Elution Buffer at 60°C. Incubate Lysis Buffer at 50°C for 5 min*

1. Cut about 0.8 cm of the mouse tail into small pieces and transfer the material into a 1.5 or 2.0 ml reaction tube.
2. If possible, the material can also be grinded under liquid nitrogen, so increasing the lysis efficiency.
3. Add 300 µl of **Lysis Buffer G** and 20 µl **Proteinase K**, incubate at 60°C for lysis of the material (continuous shaking for instance in a thermomixer increases lysis efficiency).
4. Centrifuge for 1 min at 12 - 14.000 rpm for pelleting particles, which are not lysed. Transfer the supernatant into a new reaction tube (1.5 or 2.0 ml)

Note: *The Binding Buffer G has to be vortexed prior to use!*

5. Addition of 600 µl **Binding Buffer G** and mix briefly and powerfully.
6. Incubate the reaction tube at room temperature for 3 min.
7. Centrifuge for 30 sec at 10.000 rpm (short spin) to pellet down the silica particles; then discard the supernatant carefully.
8. Add 1 ml Wash Buffer, resuspend the Silica particles pellet by pipetting up and down or by brief manual until the pellet is completely resuspended; then centrifuge at 30 sec at 10.000 rpm (short spin). Discard the supernatant carefully.
9. Repeat the step 7 once again, then briefly spin down the residual fluid and remove the residual Wash Buffer as completely as possible by pipetting. Then incubate the open tube in a Vacuum dessicator or a water bath at 60°C for some minutes to completely evaporate the residual ethanol from the Wash Buffer (Thermomixer or Thermal cycler can also be used).

   Note: *Strictly avoid overdrying of the Silica particles pellet!*

10. Addition of 200 µl **Elution Buffer** or Tris buffered ddH_2O (pH 8.5 - 9.0) prewarmed to 60°C. Thoroughly resuspend the pellet by pipetting up and down.
11. Incubate at 60°C for 3 min.
12. Centrifuge at 14.000 rpm for 1 min and transfer the DNA containing supernatant into a new reaction tube. Be very careful while pipetting the supernatant to avoid carry-over of silica particles (leave at least 2 µl supernatant above the pellet).

Note: *Regardless of how carefully the final DNA solution was separated from the Silica pellet, some residual particles always remain in the solution, which may affect the activity of DNA-modifying enzymes. To avoid associated problems, we recommend centrifugation of the DNA solution for 2 min at full speed, each time prior to use. A DNA aliquot for further use should be taken immediately after centrifugation from the top of the solution.*

Troubleshooting

Problem/probable cause	Comments and suggestions
low yield of extracted DNA	
ineffective destruction of starting material	mechanical pre cutting of the material, or grinding under liquid nitrogen
	increasing of lysis times
improper storage of starting material	store the samples carefully.
poor DNA binding by Silica particles	mix the sample with the Silica particles carefully
substantial loss of silica particles-bound DNA in the washing step due to low ethanol concentration in the Wash Buffer	prepare the Wash Buffer exactly as described in the manual
poor elution of the DNA bound on the Silica particles	storge of Wash Buffer with firmly fixed cap
	avoid over drying of the pellet
	prewarm Elution Buffer to 60°C
bad results in PCR	
contamination of final DNA solution by chaotropic salts	washing of the Silica particles pellet thoroughly as described in manual
contamination of final DNA solution by particles of Silica particles	before using the DNA centrifuge the solution for 2min
contamination of final DNA solution by ethanol	remove the ethanol carefully, test the smell
poor cleavage byrestriction endo-nucleases or incomplete digestions of the purified genomic DNA	
see at “bad PCR products“	

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® Genomic Kit II	500 preparations	1032120400
Related products		
Invisorb® Spin Tissue Mini Kit	250 preparations	1032100300

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