Instructions for use
Invisorb® Spin Tissue Mini Kit

REF 1032100200
1032100300

Σ 50 preparations
250 preparations

Invitek Molecular GmbH
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Germany

INVITEK
Molecular

Important notes

Thank you for purchasing the **Invisorb® Spin Tissue Mini Kit** from Invitek Molecular.

The product serves the purpose of manual isolation of DNA from human and animal tissue (e.g. muscle, liver, heat, brain, rodent tail), insects, biopsy material, eukaryotic cells, buccal swabs and food samples of animal origin, using Spin Column technology.

WARNING! Improper handling and use for other than the intended purpose can cause danger and damage. Therefore, we ask you to read through these instructions for use and follow them carefully. Always keep them handy. To avoid personal injury, also observe the safety instructions.

All versions of the instructions for use can be found on our website for download or can be requested from us: www.invitek-molecular.com

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1. Safety instructions

Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- When and while working with chemicals, always wear protective clothing, disposable gloves and safety glasses.
- Always change pipette tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipette tips.
- Do not reuse any consumables.
- 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 airflow until the samples are lysed.

Before handling chemicals read and understand all applicable safety data sheets (MSDS). These are available online at www.invitek-molecular.com.

Dispose of kit residues and waste fluids in accordance with your country's regulations, again refer to the MSDS. Invitek Molecular has not tested the liquid waste generated by the kit 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 must be handled and disposed of according to local safety regulations.

European Community risk and safety phrases for the components of the **Invisorb® Spin Tissue Mini Kit** 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, www.infotrac.net:

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

2. Product information

2.1 Kit contents

	50 purifications	250 purifications
Catalogue No.	1032100200	1032100300
Lysis Buffer G	30 ml/bottle	120 ml/bottle
Binding Buffer A	1 x 4 ml/bottle (final volume 1 x 15 ml)	2 x 9 ml/bottle (final volume 2 x 30 ml)
Proteinase S	2 x 2 ml/vial	6 x 2 ml/vial
Wash Buffer	1 x 18 ml/bottle (final volume 1 x 60 ml)	2 x 45 ml/bottle (final volume 2 x 150 ml)
Elution Buffer	30 ml/bottle	120 ml/bottle
Spin Filter	1 x 50 pieces	5 x 50 pieces
2.0 ml Receiver Tubes	1 x 50 pieces	5 x 50 pieces
1.5 ml Receiver Tubes	1 x 50 pieces	5 x 50 pieces
Short Protocol	1 leaflet	1 leaflet

2.2 Reagents and equipment to be supplied by user

Lab equipment:

- Microcentrifuge (*all protocols were validated with a Centrifuge 5415 D Eppendorf)*
- Optional: centrifuge for 15 or 50 ml
- Thermoshaker
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipette and pipette tips
- Vortex mixer
- Reaction tubes (1.5 ml, 2.0 ml)

Liquids and solvents:

- 96 - 100 % ethanol (non-denatured)
- Isopropanol*
- Optional (for RNA removal): RNase A (10 mg/ml)

*The 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

2.3 Storage, appearance, and shelf life

Shelf life: All buffers and kit components should be stored at room temperature and have a shelf life as indicated on the outer kit package label.

After opening, individual components of the kit, as well as components prepared accordingly before first use, have a shelf life of 3 months.

Before each use, make sure that all components are at room temperature. If there are temperature-related precipitates in the solutions, dissolve them by carefully warming (up to 30°C).

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

Wash Buffer: after adding ethanol, it should be firmly closed and stored at room temperature.

Binding Buffer A: after adding isopropanol, it should be firmly closed and stored at room temperature.

Proteinase S is coloured blue, making it easier to follow the transfer of small amounts of enzyme.

2.4 Intended use

The **Invisorb® Spin Tissue Mini Kit** is a Spin Column technology based nucleic acid extraction kit, intended for the manual isolation and purification of genomic DNA.

The kit can be used for a variety of sample types, such as fresh or frozen human and animal tissue (e.g. muscle, liver, heat, brain, rodent tail) insects, biopsy material, eukaryotic cells, buccal swabs and food samples of animal origin.

The product is intended for use by professionals only, such as laboratory technicians, physicians and biologists trained in molecular biological techniques and *in vitro* diagnostic procedures.

2.5 Product information and specifications

Starting material	Yield	Quality	Time
0.5 - 40 mg tissue sample	Tissue: up to 50 µg (depending on type and sample volume)	A_{260} : A_{280} 1.6 – 2.1	approx. 15 min for 12 samples (excl. lysis)
Up to 2 mg Biopsy material	Biopsy material: 0.2 - 1.5 µg per mg tissue		
Up to 1.0 cm mouse tail Up to 0.5 cm rat tail	Rodent tail: 10-40 µg per tail		
10 - 10^6 eukaryotic cells	Cells: up to 10 µg		
Buccal swabs	Buccal swabs: up to 2 µg		

Yield and quality of purified nucleic acids depend on the sample type, sample source, transport, storage and age.

The kit can also be used for food samples of animal origin. The yield and quality of isolated DNA from food samples depends on the type of food and especially on the degree of processing. Heavy processing of the food can lead to degradation of nucleic acids, therefore a lower DNA yield and fragmented DNA can be expected in this case.

The kit isolates all types of DNA from the sample material that are longer than 50 base pairs: Nucleic DNA, mitochondrial DNA, degraded DNA, fragmented DNA as well as apoptotic DNA.

The kit uses gentle, non-chaotropic chemicals, for the isolation of intact, highly pure DNA.

Downstream Applications:
Yield and quality of isolated nucleic acids are in general suitable for plenty of molecular applications such as PCR techniques, NGS and hybridization methods. Downstream applications should be performed according to the respective manufacturers' specifications.

2.6 Principle and procedure

1. Lyse samples

Samples are lysed under non-chaotropic conditions at elevated temperature. Lysis is performed with Lysis Buffer G and Proteinase S. Proteinase S is coloured blue to follow the addition of the small volume of enzyme.

Prior to lysis, the samples must be prepared according to the sample type, e.g., by grinding tissue with liquid nitrogen to increase lysis efficiency.

2. Bind DNA

By adding Binding Buffer A to the lysate, optimal binding conditions are adjusted. Each lysate is then applied to a Spin Filter and nucleic acids are adsorbed to the membrane.

3. Wash to remove residual contaminations

Contaminants are efficiently washed away using Wash Buffer, while the genomic DNA remains bound to the membrane.

4. Elute DNA

DNA is eluted from the Spin Filter using 50 - 200 µl Elution Buffer. The elution volume depends on the DNA content of the starting material and the desired DNA concentration.

3. Nucleic acid extraction with the Invisorb® Spin Tissue Mini Kit

3.1 Before starting a protocol

When using the kit for the first time make sure all buffers and reagents are prepared as indicated:

Buffer preparations prior first use: 50 preparations
Binding Buffer A: Add 11 ml **99.7% isopropanol** (molecular biologic grade) to the bottle. Mix by inverting for 1 min. Shortly before use mix by inverting several times. Always keep the bottle firmly closed. **Wash Buffer:** Add 42 ml of **96 - 100% ethanol** to the bottle. Mix thoroughly, always keep the bottle firmly closed.
Buffer preparations prior first use: 250 preparations
Binding Buffer A: Add 21 ml **99.7% isopropanol** (molecular biologic grade) to each bottle. Mix by inverting for 1 min. Shortly before use mix by inverting several times. Always keep the bottles firmly closed. **Wash Buffer:** Add 105 ml of **96 - 100% ethanol** to each bottle. Mix thoroughly, always keep the bottles firmly closed.

- Adjust the thermoshaker to 52°C.
- Warm up the needed amount of **Elution Buffer** to 52°C (50 - 200 µl **Elution Buffer** are needed per sample).
- Determine the number of required reactions including controls and label the needed amount of Spin Filters and the needed amount of 2.0 ml Receiver Tubes and 1.5 ml Receiver Tubes (per sample: 1 Receiver Tube is needed).
- place the Spin Filter into labelled 2.0 ml Receiver Tubes

3.2 Sampling and storage of starting material

For reproducible and high yields, the correct sample storage is essential. Yields may vary depending on factors such as health of the donor, sample age, sample type, transport and storage.

Repeated freeze-thaw cycles of samples should be avoided to prevent nucleic acid degradation. In general, best results are obtained using fresh samples.

Tissue, biopsy material, rodent tail: Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C.

After Lysis and Proteinase S digestion, tissue samples can be stored in Lysis Buffer G/Proteinase S for up to 6 months at –20°C without affecting DNA quality.

Insects: Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C.

Cells: Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C

Buccal swabs: Please collect samples according to the manufacturer's instructions. Alternatively, the following procedure is recommended for sample collection for all common swabs: swipe the swab firmly against the inside of each cheek 6 times. Allow the swab to air dry after collection or use freshly. Make sure that nothing is eaten or drunk in the 30 minutes prior to sampling. Store dry at 4-8°C.

Food: For sample collection, use clean, dry, leak-proof, wide-mouth containers suitable for the product samples. Transport refrigerated samples at 0 - 4°C, use a sample transport box with suitable refrigerant so that the sample can be kept at 0 - 4°C until arrival at the laboratory. Do not freeze refrigerated products. Unless otherwise specified, refrigerated samples should not be analysed more than 36 hours after collection.

3.3 Preparation of starting materials

In the following the preparation of the sample lysis for different starting materials is described.

3.3.1 Tissue, biopsy material, rodent tail

Consider the DNA content of the tissue type for the sample volume used for extraction. In general, 0.5 – 40 mg of tissue or up to 1.0 cm mouse tail sample can be extracted. An excessive amount of sample material may result in inefficient lysis. For DNA-rich tissue, such as liver, it is recommended to use only 20 mg of sample material for DNA extraction. If the DNA content is not known, it is recommended to use less than 40 mg sample. On average, 5-40 µg of DNA can be extracted from 25 mg of tissue material.
Optimal digestion of the tissue is important to obtain maximum yield and purity of genomic DNA. For disruption and homogenization of the sample, grinding with a mortar and pestle or, for example, a rotor-star homogenizer can be used. For disruption of difficult to lyse tissue like cartilage, kidney, and heart muscle, bead beating with Zirconia beads (available separately) is recommended.

Careful disruption of the tissue may shorten the incubation time in Lysis Mixture (Lysis Buffer G incl. Proteinase S). It is also possible to disrupt the sample directly in Lysis Mixture to achieve good homogenization.

3.3.2 Insects

Insects, especially those with chitinous bodies, must be homogenized well before lysis (e.g., by grinding with a mortar and pestle). The sample can be stored for a short time at 2 - 8°C in Lysis Buffer G. Chitinase may help with some insects with a strong armour.

3.3.3 Cells

Take care to remove culture media completely. Incomplete removal dilutes the lysate and affects lysis efficiency.

a) Cells grown in suspension

Centrifuge up to 1 x 10^6 cells for 5 min at 300 x g. Discard the supernatant and completely remove the medium, taking care not to disturb the cell pellet. Cells can now be frozen (at -80°C) or used immediately for lysis.

For sample lysis add 400 µl Lysis Buffer G and 40 µl Proteinase S to the pellet and vortex thoroughly. Transfer the complete mixture into a 1.5 ml reaction tube (not provided)

Proceed as described in the extraction protocol step 3.

b) Cells grown in a monolayer

Remove the medium completely from the cells and proceed immediately with the lysis step. Alternatively, cells can be separated by trypsinization (cultivation in larger culture vessels, such as dishes >35 mm, flasks >12.5 cm^2). Transfer cells to a 50 ml reaction tube, pellet by centrifugation at 300 x g for 5 min and discard the supernatant completely. Cells can now be frozen (at -80°C) or used immediately for lysis.

For sample lysis add 400 µl Lysis Buffer G and 40 µl Proteinase S to the pellet and vortex thoroughly. Transfer the complete mixture into a 1.5 ml reaction tube (not provided)

Proceed as described in the extraction protocol step 3.

3.3.4 Buccal swabs

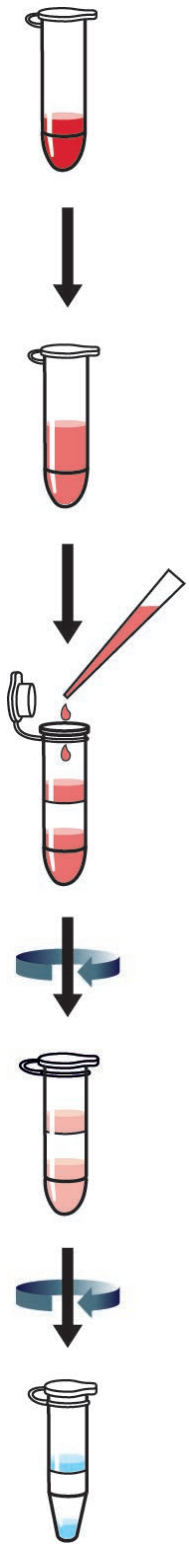
Transfer 400 µl **Lysis Buffer G** and 40 µl **Proteinase S** into a 1.5 ml Reaction Tube (not provided).
Rinse the swab in the prepared tube. Carefully squeeze the swab on the inner wall of the tube to obtain as much sample as possible and discard the swab afterwards.

Proceed as described in the extraction protocol step 3.

3.3.5 Food samples of animal origin

Homogenize a representative amount of sample with a commercial homogenizer and transfer 40-100 mg of homogenate into a 1.5 ml Reaction Tube (not provided) to proceed with the lysis.

3.4 Short protocol Invisorb® Spin Tissue Mini Kit



Lyse samples

Refer to chapter 3.3 “Preparation of starting material” for sample specific pre-treatment.

Note: For processing of food samples a centrifugation speed of 13.500 x g can be used throughout the protocol.

1. After pre-treatment, transfer the starting material to a 1.5 ml reaction tube (not provided).
2. Add **400 µl Lysis Buffer G** and **40 µl Proteinase S**, vortex thoroughly.
3. Incubate the reaction tube at 52°C constantly shaking until lysis is complete (in average 20 -30 min, depending on sample type. Do not exceed a maximum lysis time of 16 h). For sample material that is difficult to lyse vortex from time to time.
4. Centrifuge for 2 min at 11.000 x g 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, add 40 µl **RNase A** (10 mg/ml), vortex shortly and incubate for 5 min at RT.

Bind nucleic acids

5. Add **200 µl Binding Buffer A** and vortex for 10 sec.
6. Place a Spin Filter in a 2.0 ml Receiver Tube.
   Transfer the reaction mixture into the Spin Filter and incubate for 1 min.
   Close the Spin Filter and centrifuge at 13.500 x g for 3 min.
7. Discard the filtrate and place the Spin Filter back to the Receiver Tube.

Wash to remove residual contaminations

8. Add **550 µl Wash Buffer**, close the Spin Filter and centrifuge at 11.000 x g for 1 min. Discard the filtrate, place the Spin Filter back to the Receiver Tube.
9. Add **550 µl Wash Buffer**, close the Spin Filter and centrifuge at 11.000 x g for 1 min. Discard the filtrate, place the Spin Filter back to the Receiver Tube.
10. Centrifuge at maximum speed for 4 min to remove residual Ethanol.

Elute nucleic acids

11. Place the Spin Filter into a 1.5 ml Receiver Tube and add 50-200 µl **Elution Buffer** (preheated to 52°C) directly onto the Spin Filter. Incubate for 3 min at room temperature. Centrifuge for 1 min at 11.000 x g.

3.5 Protocol: DNA isolation from tissue, biopsy sample, rodent tail, insects, cells, buccal swabs, and food samples of animal origin

Please refer to chapter 3.3 “Preparation of starting material” for sample specific pre-treatment.

Note: *For processing of food samples a centrifugation speed of 13.500 x g can be used throughout the protocol.*

Note: ***Liver and other DNA rich tissue samples:*** *take not more than 20 mg for DNA isolation.*
Rodent tail: *up to 1.0 cm can be used as starting material*

1. Transfer the starting material into a 1.5 ml reaction tube (not provided). Depending on the starting material mechanical disruption or cutting the material is required (please refer to chapter 3.3)
2. Add **400 µl Lysis Buffer G** and **40 µl Proteinase S** and vortex thoroughly.
3. Incubate the reaction tube at 52°C, constantly shaking until lysis is complete. The average lysis time is 20-30 min, depending on the sample type. Overnight lysis is possible, 16 h should not be exceeded. For difficult to lyse sample material vortex from time to time.
4. Centrifuge for 2 min at 11.000 x g 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, add **40 µl 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 reaction mixture onto the Spin Filter and incubate for 1 min.
   Close the Spin Filter and centrifuge at 13.500 x g for 3 min.
7. Discard the filtrate and place the Spin Filter back to the Receiver Tube.
8. Add **550 µl Wash Buffer**, close the Spin Filter and centrifuge at 11.000 x g for 1 min. Discard the filtrate, place the Spin Filter back to the Receiver Tube.
9. Add **550 µl Wash Buffer**, close the Spin Filter and centrifuge at 11.000 x g for 1 min. Discard the filtrate, place the Spin Filter back to the Receiver Tube.
10. Centrifuge at maximum speed for 4 min to remove the residual Ethanol.
11. Place the Spin Filter into a 1.5 ml Receiver Tube and add 50-200 µl **Elution Buffer** (preheated to 52°C) directly onto the Spin Filter. Incubate for 3 min at room temperature. Centrifuge for 1 min at 11.000 x g. Discard the Spin Filter.

Note: *The elution volume depends on the DNA content of the starting material and the desired DNA concentration. The elution volume should not be less than 50 µl to avoid yield losses.*

4. Appendix

4.1 Troubleshooting

Problem	Possible cause	Recommendation
Low amount of nucleic acids	Insufficient cell lysis	Increase lysis time with **Lysis Buffer G**. Continuous shaking improves lysis efficiency. Reduce amount of starting material to avoid column overload. Homogenize/cut sample material thoroughly before lysis.
	Incomplete elution	Increase incubation time with preheated **Elution Buffer** to 5-10 min. Elute twice with 100 µl **Elution Buffer**. Use a higher volume of **Elution Buffer**.
	Water used instead of **Elution Buffer**	When eluting with deionized water, ensure that the pH of the water is at least 7.0 and not below. Use 10 mM TrisHCl pH 7.5 instead of water.
	Incorrect storage of starting material	Ensure that starting material is appropriately stored. Avoid repeated thaw-freeze cycles of the sample material.
	Wash Buffers were incorrectly prepared	Ensure, that the correct amount of ethanol is added to the Wash Buffer and that all solutions are stored firmly closed.
	Insufficient mixing with **Binding Buffer A**	Mix sample with **Binding Buffer A** by pipetting or by vortexing prior transfer to the Spin Filter
Degraded nucleic acids	Incorrect storage of starting material	see above remark about incorrect storage of starting material.
	Old material	Ensure that the starting material is stored at appropriate conditions (–20°C/-80°C).
DNA does not perform well in downstream applications (e.g. real-time PCR or NGS)	Ethanol carryover during elution	Increase time of drying step for removal of ethanol.
	Salt carry-over during elution	Check the **Wash Buffers** for salt precipitates. If there are any precipitates visible, solve them by carefully warming up to 30°C. Ensure that the **Wash Buffers** are at room temperature before use.
Clogged Spin Filter	Insufficient cell lysis	See above remark about insufficient cell lysis. Increase centrifugation time/speed. Centrifuge after lysis to pellet un-lysed material. If you observe a gelatinous pellet after incubation at 52°C increase volume of **Proteinase S** to 60 µl and prolong lysis time.
A_{260}:A_{280} ratio is too low/high	High level of residual RNA	Perform **RNAse A** treatment before the binding step.

4.2 Warranty

Invitek Molecular guarantees the correct function of the kit for applications described in this manual and in accordance with the intended use. In accordance with Invitek Molecular's EN ISO 13485 certified Quality Management System the performance of all kit components has been tested to ensure product quality.
Any problems, incidents or defects shall be reported to Invitek Molecular immediately upon detection. Immediately upon receipt, inspect the product to ensure that it is complete and intact. In the event of any discrepancies, you must inform Invitek Molecular immediately in writing. Modifications of the kit and protocols and use that deviate from the intended purpose are not covered by any warranty.

Invitek Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.
Invitek Molecular warrants products as set forth in the General Terms and Conditions available at www.invitek-molecular.com. If you have any questions, please contact techsupport@invitek-molecular.com.

4.3 Symbols used on product and labelling

 Manufacturer

 Lot number

 Catalogue number

 Expiry date

 Consult operating instructions

 Temperature limitation

 Do not reuse

 Amount of sample preparations

4.4 Further documents and supplementary information

Visit www.invitek-molecular.com for further information on:

- FAQs and troubleshooting tips
- Manuals in different languages
- Safety data Sheets (MSDS)
- Web support
- Product videos

If, despite careful study of the operating instructions and further information, you still require assistance, please contact us at techsupport@invitek-molecular.com or the dealer responsible for you.

4.5 Ordering information

Product	Package Size	Catalogue No.
Invisorb® Spin Tissue Mini Kit	50 preparations	1032100200
Invisorb® Spin Tissue Mini Kit	250 preparations	1032100300

Revision history

Revision	Date	Description
EN-v1-2022	2022-12-08	New document

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2022-12-08 EN-v1-2022