Instructions for use

InviSorb® Spin Plant Mini Kit

INVITEK diagnostics

InviSorb®

Language: EN

REF 1037100200
1037100300

Σ 50 preparations
250 preparations

Invitek Molecular GmbH
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Germany

Important notes

Thank you for purchasing the **InviSorb® Spin Plant Mini Kit** from Invitek Diagnostics.

The product serves the purpose of manual isolation of DNA from a wide range of plant species and materials (fresh, frozen or dried plant material from e.g. leaves, roots, fruits or seeds) and food samples of plant 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.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.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 Plant Mini Kit** to which they apply are listed below as follows:

Proteinase S

Danger

Hazard statements
H318 - Causes serious eye damage.
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Precautionary statements
P261 - Avoid breathing dust/fume/gas/mist/vapours/spray.
P284 - Wear respiratory protection.
P304+P340 - IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P305+P351+P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 - Immediately call a POISON CENTER or doctor.
P342+P311 - If experiencing respiratory symptoms: Call a POISON CENTER or doctor.
P501 - Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.

Lysis Buffer P


Warning

Hazard statements
H319 - Causes serious eye irritation.
H412 - Harmful to aquatic life with long lasting effects
Precautionary statements
P273 - Avoid release to the environment.
P305+P351+P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313 - If eye irritation persists: Get medical advice/attention.
P501 - Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.

Wash Buffer I


Danger

Hazard statements
H302+H332 - Harmful if swallowed or if inhaled.
H314 - Causes severe skin burns and eye damage.
H412 - Harmful to aquatic life with long lasting effects
Precautionary statements
P260 - Do not breathe dust/fume/gas/mist/vapours/spray.
P271 - Use only outdoors or in a well-ventilated area.
P273 - Avoid release to the environment.
P301+P312 - IF SWALLOWED: Call a POISON CENTRE or doctor if you feel unwell.
P301+P330+P331 - IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303+P361+P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.
P501 - Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.

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.	1037100200	1037100300
Lysis Buffer P	30 ml/bottle	120 ml/bottle
Binding Buffer A	4 ml/bottle (final volume 15 ml)	2 x 9 ml/bottle (final volume 2 x 30 ml)
Proteinase S	2 ml/vial	6 x 2 ml/vial
Wash Buffer I	30 ml/bottle (final volume 60 ml)	80 ml/bottle (final volume 160 ml)
Wash Buffer II	18 ml (final volume 60 ml)	2 x 45 ml (final volume 2 x 150 ml)
Elution Buffer	15 ml/bottle	60 ml/bottle
Prefilter	50 pieces	5 x 50 pieces
Spin Filter	50 pieces	5 x 50 pieces
2.0 ml Receiver Tubes	2 x 50 pieces	5 x 50 pieces
1.5 ml Receiver Tubes	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)*
- Thermoshaker (65°C)
- 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: RNase A (10 mg/ml)
- Optional: Lysozyme (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 I and II: 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 Plant Mini Kit** is a Spin Column technology based nucleic acid extraction kit, intended for the manual isolation and purification of genomic DNA. Besides the isolation of genomic DNA from plant material, plant pathogens such as bacteria can be purified.

The kit can be used for a variety of sample types of plant origin, such as fresh, frozen or dried material from different plant species and materials e.g. leaves, roots, fruits or seeds. The kit also can be used for the isolation of DNA from food samples of plant 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
Up to 100 mg plant material Up to 60 mg of dried plant material	up to 50 µg (depending on sample type and volume)	A_{260} : A_{280} 1.6 – 2.0	approx. 20 min (excl. lysis)

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 plant 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 uses gentle, non-chaotropic chemicals, for the isolation of intact, highly pure DNA. The kit allows DNA purification without the use of phenol/chloroform.

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
Before lysis, samples must be homogenized, e.g., by grinding with liquid nitrogen to increase lysis efficiency.
Samples are lysed under non-chaotropic conditions at elevated temperature. Lysis is performed with Lysis Buffer P and Proteinase S. Optionally, an incubation with Lysozyme can be done to extract DNA from bacteria inside the plant tissue.
After sample lysis, residues of plant material are removed using a Prefilter.

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 I and II, while the genomic DNA remains bound to the membrane.

4. Elute DNA
DNA is eluted from the Spin Filter using 100 µl Elution Buffer. Optionally, the elution volume can be changed from 50-200 µl to adjust DNA concentration of the eluate.

3. Nucleic acid extraction with the InviSorb® Spin Plant 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 I: Add 30 ml of **96 - 100% ethanol** to the bottle. Mix thoroughly, always keep the bottle firmly closed.
Wash Buffer II: 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 I: Add 80 ml of **96 - 100% ethanol** to the bottle. Mix thoroughly, always keep the bottle firmly closed.
Wash Buffer II: Add 105 ml of **96 - 100% ethanol** to each bottle. Mix thoroughly, always keep the bottles firmly closed.

- Adjust the thermoshaker to 65°C.
- Transfer the required amount of **Elution Buffer** (100 µl **Elution Buffer** are needed per sample) to a 2.0 ml reaction tube (not provided) and prewarm to 65°C.

- 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, 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.

Plant material: samples from plant origin can be stored at room temperature for 2 – 3 hours, for short time storage (up to one week) samples may be stored at 2 - 8 °C. For long-term storage, we recommend freezing samples at –20°C or –80°C. However, some samples (e.g., tree buds) can be stored for a longer period at 4°C.

Dried sample material should be stored protected and dry at room temperature, using a closed container to prevent dehydration.

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 material is described.

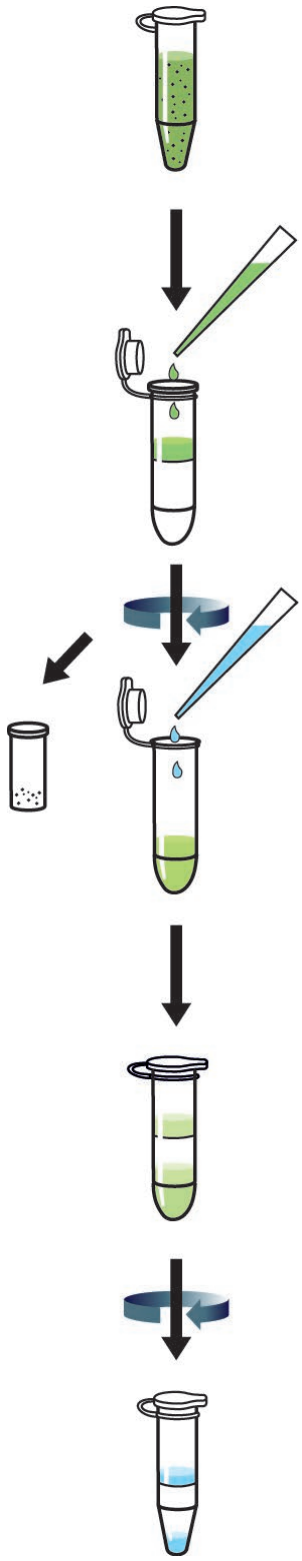
3.3.1 Plant material

For the extraction of DNA from plant material, it is important to carry out a good homogenisation in order to break down the rigid cell wall structure. For the homogenisation of leaves, roots, seeds and other plant material, mortar and pestle or a mill can be used in combination with liquid nitrogen.

3.3.2 Food samples of plant origin

Homogenize a representative amount of sample with a commercial homogenizer or laboratory blender transfer up to 100 mg of homogenate into a 1.5 ml Reaction Tube (not provided) to proceed with the lysis.

3.4 Short protocol InviSorb® Spin Pant Mini Kit



Lyse samples

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

1. Transfer up to 100 mg of homogenized starting material to a 1.5 ml reaction tube.
2. Add **400 µl Lysis Buffer P** and **20 µl Proteinase S**, vortex briefly.

   Optional: Add 20 µl Lysozyme before adding Proteinase S, vortex briefly and incubate for 10 min at 37°C.
3. Incubate the reaction mix at 65°C constantly shaking, for 30 min or longer, until lysis is complete.
4. Place the Prefilter into a 2.0 ml Receiver Tube and transfer the complete reaction mix to the Prefilter.
   Centrifuge for 2 min at 11.000 x g. Discard the Prefilter.
   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 onto the Spin Filter and incubate for 1 min.
   Close the Spin Filter and centrifuge at 11.000 x g for 2 min.
   Discard the filtrate and place the Spin Filter back to the Receiver Tube.

Wash to remove residual contaminations

7. Add **550 µl Wash Buffer I**, 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.
8. Add **550 µl Wash Buffer II**, 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. Repeat the washing step.
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 100 µl **Elution Buffer** (preheated to 65°C) directly onto the Spin Filter. Incubate for 3 min at RT. Centrifuge for 1 min at 11.000 x g. Discard the Spin Filter.

3.5 Protocol: DNA isolation from fresh, frozen or dried plant material, and food samples of plant origin

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

1. Transfer up to 100 mg of homogenized, powdered starting material to a 1.5 ml reaction tube.

   Note: *Use 120-180 mg for material with a high water content (e.g. fruits, algae).*

2. Add **400 µl Lysis Buffer P** and **20 µl Proteinase S** and vortex briefly.

   Optional: *to extract bacterial DNA add **20 µl Lysozyme** before adding Proteinase S, vortex briefly and incubate at 37°C for 10 min.*

3. Incubate the reaction mix at 65°C, constantly shaking, for 30 min or longer, until lysis is complete.

4. Place the Prefilter into a 2.0 ml Receiver Tube and transfer the complete reaction mix to the prefilter.
   Centrifuge for 2 min at 11.000 x g. Discard the Prefilter.

   Note: *To remove RNA (if necessary) from the sample, add 40 µl RNase A* (10 mg/ml) *to the filtrate, vortex briefly and incubate for 5 min at RT.*

5. Add **200 µl Binding Buffer A** and vortex thoroughly.

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 11.000 x g for 2 min.
   Discard the filtrate and place the Spin Filter back to the Receiver Tube.

7. Add **550 µl Wash Buffer I**, 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.

8. Add **550 µl Wash Buffer II**, 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. Repeat the washing step: Add **550 µl Wash Buffer II**, 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 100 µl **Elution Buffer** (preheated to 65°C) directly onto the Spin Filter. Incubate for 3 min at RT. Centrifuge for 1 min at 11.000 x g. Discard the Spin Filter.

    Note: *DNA can also be eluted with a lower or higher volume of Elution Buffer (depending on the expected yield of genomic DNA). The elution volume should not be less than 50 µl to avoid yield losses. The maximum elution volume is 200 µl Elution Buffer.*
    To maximize the final yield, a second elution step with the equal volume of Elution Buffer is recommended.

4. Appendix

4.1 Troubleshooting

Problem	Possible cause	Recommendation
Low amount of nucleic acids	Insufficient cell lysis	Increase lysis time with Lysis Buffer P. Continuous shaking improves lysis efficiency. Reduce amount of starting material to avoid column overload.
	Incomplete elution	Increase incubation time with preheated Elution Buffer to 5-10 min. Elute twice with the sample volume of Elution Buffer. Use a higher volume of Elution Buffer.
	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 Buffers 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 to transfer to the Spin Filter.
	Too much Elution Buffer	Elute with a lower volume of Elution Buffer (min. 50 µl).
Degraded nucleic acids	Old material	Ensure that the starting material is stored at appropriate conditions (–20°C/-80°C).
	Incorrect storage of starting material	See above.
DNA does not perform well in downstream applications (e.g. real-time PCR or NGS)	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.
	Ethanol carryover during elution	Increase time of drying step for removal of ethanol.
Clogged Spin Filter	Insufficient cell lysis and/or too much starting material	See above remark about insufficient cell lysis. Increase centrifugation time. Increase centrifugation speed to 13.500 x g for the whole protocol (e.g. when processing food samples). Reduce the amount of starting material.
RNA contamination	High level of residual RNA	Perform RNase A treatment as described in the protocol.

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.com. If you have any questions, please contact techsupport@invitek.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.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.com or the dealer responsible for you.

4.5 Ordering information

Product	Package Size	Catalogue No.
InviSorb® Spin Plant Mini Kit	50 preparations	1037100200
InviSorb® Spin Plant Mini Kit	250 preparations	1037100300

Revision history

Revision	Date	Description
EN-v1-2024	2024-03-13	New document

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