

Instructions for use

InviSorb® Spin Soil DNA Kit

INVITEK
diagnostics



InviSorb®

Language: EN

REF 1039100200
1039100300



50 preparations
250 preparations



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Important notes

Thank you for purchasing the **InviSorb® Spin Soil DNA Kit** from Invitek Diagnostics.

The product serves the purpose of manual isolation of DNA from microorganisms in soil samples 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 Soil DNA Kit** to which they apply are listed below as follows:

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.

Proteinase K



Danger

Hazard statements

H315 - Causes skin irritation.

H319 - Causes serious eye irritation.

H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.

H335 - May cause respiratory irritation.

Precautionary statements

P261 - Avoid breathing dust/fume/gas/mist/vapours/spray.

P284 - Wear respiratory protection.

P302+P352 - IF ON SKIN: Wash with plenty of water.

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.

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

Contains: guanidinium thiocyanate

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
Catalog No.	1039100200	1039100300
InviAdsorb	50 tubes	5 x 50 tubes
Zirconia Beads II	2 vials	8 vials
Lysis Buffer P	180 ml/bottle	2 x 180 ml/bottle
Proteinase K	1 vial for 1.5 ml working solution	5 vials for 5 x 1.5 ml working solution
Binding Buffer A	9 ml/bottle (final volume 30 ml)	36 ml/bottle (final volume 120 ml)
Wash Buffer I	30 ml/bottle (final volume 60 ml)	80 ml/bottle (final volume 160 ml)
Wash Buffer II	2 x 18 ml/bottle (final volume 60 ml)	3 x 45 ml/bottle (final volume 3 x 150 ml)
Elution Buffer	15 ml/bottle	60 ml/bottle
2.0 ml Safe-Lock-Tubes	2 x 50 pieces	2 x 250 pieces
RTA Spin Filter Set	50 sets	250 sets
RTA Receiver Tubes	2 x 50 pieces	2 x 250 pieces
1.5 ml Receiver Tubes	2 x 50 pieces	2 x 250 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
- Thermo shaker (37°C - 95°C)
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipette and pipette tips
- Vortex mixer
- Reaction tubes (1.5 ml, 2.0 ml)

Liquids and solvents:

- DNase/RNase free water
- 96 - 100 % ethanol (non-denatured)
- Isopropanol*

*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 Wash Buffer II: after adding ethanol, they 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 K: once dissolved in DNase/RNase free water Proteinase K can be stored at 2 - 8 °C for up to two months. For longer storage keep at –20 °C, freeze-thaw once only.

Lysis Buffer P: If there are temperature-related precipitates, dissolve them by carefully warming at 30°C in a water bath, shake occasionally during the dissolution process.

2.4 Intended use

The **InviSorb® Spin Soil DNA Kit** is a Spin Column technology based nucleic acid extraction kit, intended for the isolation and purification of microbial DNA from soil samples. Related sample materials such as degraded plant material or sand can also be used for sample extraction.

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

2.5 Product information and specifications

Starting material	Yield	Quality	Time
max. 200 mg soil sample	Up to 10 µg, (depending on sample material)	A ₂₆₀ : A ₂₈₀ 1.4 – 1.8	approx. 45 min (incl. lysis)

Yield and quality of purified DNA is depending on microorganism content, sample source, and storage. Soil samples vary greatly in their nature and composition. Humic acid content, the proportion of plant degradation products and many other parameters influence the microbial composition and thus the DNA yield. A typical yield is 1 – 10 µg.

Downstream Applications:

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

2.6 Principle and procedure

1. Lyse samples

Soil samples are lysed in Lysis Buffer P under denaturing conditions. Biological material of plant or animal source, bacterial cells and those of other microorganisms are efficiently lysed by incubation at room temperature. For bacteria that are difficult to lyse (e.g., gram-positive bacteria), an optional heating step can be performed. Additionally, Zirconia beads are added to increase lysis efficiency.

2. Removal of PCR inhibitors and protein digestion

After lysis, DNA degrading substances and PCR inhibitors present in the soil are adsorbed to the InviAdsorb matrix. InviAdsorb is in pre-filled Safe-Lock-Tubes into which the lysate must be placed. The bound contaminants and cell debris are pelleted by centrifugation. The supernatant contains the pre-purified DNA.

Proteinase K is added to the supernatant to digest and degrade proteins at elevated temperature.

3. Bind nucleic acids

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

4. Wash to remove residual contaminations

Contaminants are efficiently washed away using Wash Buffer I and Wash Buffer II, while nucleic acids remain bound to the membrane.

5. Elute DNA

Nucleic acids are eluted from the RTA Spin Filter using 50 - 100 µl Elution Buffer.

3. Nucleic acid extraction with the InviSorb® Spin Soil DNA 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 21 ml 99.7% isopropanol (molecular biology grade) into the bottle, mix by shaking vigorously for 1 minute. Mix by inverting several times just before use. Always keep the bottle firmly closed.
Proteinase K: Resuspend lyophilized Proteinase K by addition of 1.5 ml DNase/RNase free water to the vial, mix thoroughly until completely dissolving.
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 each bottle. Mix thoroughly, always keep the bottle firmly closed.
Buffer preparations prior first use: 250 preparations
Binding Buffer A: Add 84 ml 99.7% isopropanol (molecular biology grade) into the bottle, mix by shaking vigorously for 1 minute. Mix by inverting several times just before use. Always keep the bottle firmly closed.
Proteinase K: Resuspend lyophilized Proteinase K by addition of 1.5 ml DNase/RNase free water to each vial, mix thoroughly until completely dissolving.
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 bottle firmly closed.

- Adjust thermo shaker/heating blocks to 60°C (optionally to 90°C for hard to lyse bacteria)
- Warm up the needed amount of Elution Buffer to 60°C (50 - 100 µl Elution Buffer are needed per sample).
- Determine the number of required reactions and label the needed amount of RTA Spin Filters (lid) and the needed amount of 1.5 ml Receiver Tubes (per sample: 1 Receiver Tube is needed).

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, soil type, transport and storage.

Soil samples typically contain many compounds that can degrade DNA and inhibit downstream enzymatic reactions. For storage freezing the sample at -20°C is recommended.

3.3 Preparation of starting materials

For pipetting of liquid (muddy) samples, you may cut off the end of the pipet tip to make pipetting easier when transferring the sample into the 2.0 ml Safe-Lock-Tube, also wide bore tips can be used.

If the sample is frozen, use a scalpel or spatula to scrape bits of soil into the 2.0 ml Safe-Lock-Tube on ice. Take care that samples do not thaw until Lysis Buffer P is added, otherwise the DNA in the sample may degrade.

Complete homogenization during sample lysis is important for DNA isolation efficiency. If the soil type requires it, it is recommended to apply the zirconia beads with a vortex mixer or another device with strong mixing of the sample (such as FastPrep® Homogenizer - MP Bio or Bullet Blender® Homogenizer - Next Advance).

3.4 Short protocol InviSorb® Spin Soil DNA Kit

Lyse samples

1. Weigh 200 mg of soil sample (fresh or frozen) into a 2.0 ml Safe-Lock-Tube, add 1.2 ml **Lysis Buffer P**, vortex vigorously for 1 min.

Incubate 10 min at RT continuously shaking at 900 rpm.

Add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min at RT.

Centrifuge for 1 min at 11.000 x g

Adjust thermo shaker/heating block to 60°C and preheat the required amount of Elution Buffer.

Note: For difficult to lyse bacteria (e.g., gram-positive bacteria), an optional heating step can be performed, please refer to chapter 4.1 Trouble shooting in the IFU.

Removal of PCR inhibitors and protein digestion

2. Transfer the supernatant into an **InviAdsorb-Tube**, vortex vigorously for 15 sec.
Incubate for 1 min at RT. Centrifuge for 3 min at full speed.
3. Transfer the supernatant completely into a new 1.5 ml Receiver Tube, discard the pellet with the InviAdsorb. Centrifuge for 3 min at full speed.
4. Transfer 25 µl **Proteinase K** into a new 2.0 ml Safe-Lock-Tube, pipette 400 µl of the supernatant from step 3 into the Safe-Lock-Tube containing **Proteinase K**, mix shortly by vortexing. Incubate for 10 min at 60°C continuously shaking at 900 rpm.

Bind genomic DNA

5. Add 200 µl **Binding Buffer A** to the lysate, mix shortly by vortexing or by pipetting up and down several times.
Take an RTA Spin Filter Set. Transfer the mixture completely to the RTA Spin Filter.
Close the RTA Spin Filter and incubate for 1 min at RT.
Centrifuge for 2 min at 11.000 x g. Discard the filtrate and place the RTA Spin Filter in a new 2.0 ml RTA Receiver Tube.

Wash to remove residual contaminations

6. Add 500 µl **Wash Buffer I**, centrifuge for 1 min at 11.000 x g. Discard the filtrate and place the RTA Spin Filter in a new 2.0 ml RTA Receiver Tube.
7. Add 700 µl **Wash Buffer II**, centrifuge for 1 min at 11.000 x g.
Discard the filtrate and place the RTA Spin Filter **back to** the 2.0 ml RTA Receiver Tube.
8. Add 700 µl **Wash Buffer II**, centrifuge for 1 min at 11.000 x g.
Discard the filtrate and place the RTA Spin Filter **back to** the 2.0 ml RTA Receiver Tube.
9. Centrifuge for 4 min at maximum speed to eliminate the ethanol completely.
Discard the RTA Receiver Tube.

Elute DNA

10. Place the RTA Spin Filter in a 1.5 ml Receiver Tube. Add 100-200 µl of the preheated (60°C) **Elution Buffer**.
Incubate 1 min at RT.
Centrifuge for 1 min at 11.000 x g to elute DNA. Discard the RTA Spin Filter.



3.5 Protocol: Isolation of DNA from up to 200 mg soil samples

1. Weigh 200 mg of soil sample (fresh or frozen) into a 2.0 ml Safe-Lock-Tube and add 1.2 ml **Lysis Buffer P** to each sample. Vortex vigorously for 1 min. Also, for smaller sample volumes use the same amount of **Lysis Buffer P**.

Incubate 10 min at RT continuously shaking at 900 rpm.

Add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min at RT.

Centrifuge for 1 min at 11.000 x g to pellet solid soil particles and beads.

Note: *For difficult to lyse bacteria (e.g., gram-positive bacteria), an optional heating step can be performed, please refer to chapter 4.1 trouble shooting.*

2. Transfer the supernatant into an **InviAdsorb-Tube** and vortex vigorously for 15 sec.
Incubate for 1 min at RT.
Centrifuge for 3 min at full speed.
3. Transfer the supernatant completely into a new 1.5 ml Receiver Tube, discard the pellet with the InviAdsorb.
Centrifuge for 3 min at full speed.
4. Transfer 25 µl **Proteinase K** into a new 2.0 ml Safe-Lock-Tube and pipet 400 µl of the supernatant from step 3 into the Safe-Lock-Tube containing **Proteinase K**, mix shortly by vortexing.
Incubate for 10 min at 60°C continuously shaking at 900 rpm.
5. Add 200 µl **Binding Buffer A** to the lysate and mix shortly by vortexing or by pipetting up and down several times.
Take a RTA Spin Filter Set. Transfer the mixture completely into the RTA Spin Filter.
Close the RTA Spin Filter and incubate for 1 min at RT.
Centrifuge for 2 min at 11.000 x g.
Discard the filtrate and place the RTA Spin Filter in a new 2.0 ml RTA Receiver Tube.
6. Add 500 µl **Wash Buffer I** and centrifuge for 1 min at 11.000 x g.
Discard the filtrate and place the RTA Spin Filter **to a new** 2.0 ml RTA Receiver Tube.
7. Add 700 µl **Wash Buffer II** and centrifuge for 1 min at 11.000 x g.
Discard the filtrate and place the RTA Spin Filter **back to** the 2.0 ml RTA Receiver Tube.
8. Add 700 µl **Wash Buffer II** and centrifuge for 1 min at 11.000 x g.
Discard the filtrate and place the RTA Spin Filter **back to** the 2.0 ml RTA Receiver Tube.
9. Centrifuge for 4 min at maximum speed to eliminate the ethanol completely.
Discard the RTA Receiver Tube.
10. Place the RTA Spin Filter in a 1.5 ml Receiver Tube. Add 50-100 µl of the preheated (60°C) **Elution Buffer**.
Incubate 1 min at RT.
Centrifuge for 1 min at 11.000 x g to elute DNA. Discard the RTA Spin Filter.

Note: *DNA can also be eluted in a lower volume of Elution Buffer (depending on the expected yield). The minimum volume for elution is 30 µl, note that using a low elution volume can reduce the maximum yield. If a large amount of DNA is expected, the volume of elution can also be increased.*

Note: *For long-term storage, it is recommended to keep the eluted DNA at –20°C.*

4. Appendix

4.1 Troubleshooting

Problem	Possible cause	Recommendation
Clogged RTA Spin Filter	Insufficient cell lysis and/or too much starting material	Increase centrifugation speed. Reduce amount of starting material.
RNA contamination	The RTA Spin Filter can purify low amounts of RNA	Add 20 µl RNase A (10 mg/ml) and incubate for 10 minutes before adding Binding Buffer A.
Brownish Eluates	Copurification of inhibitors	Possibly, a post-purification of the eluate via a Sephadex G50 (Cytiva) column can help.
Low amount of nucleic acids	Incomplete elution	Increase incubation time with preheated Elution Buffer to 5 min. Elute twice with 50 or 100 µl Elution Buffer . Use higher volume of Elution Buffer .
	Low nucleic acid-concentration in the sample	Elute the DNA with a lower volume of Elution Buffer , do not use volumes below 30 µl.
	Incorrect storage of starting material	Ensure that starting material is appropriately stored. Avoid repeated thaw-freeze cycles of the sample material.
	Buffers were incorrectly prepared	Ensure, that the correct amount of ethanol/isopropanol is added to the Buffers and that all solutions are stored firmly closed.
	Insufficient homogenization of sample and Lysis Buffer P	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample in Lysis Buffer P until the sample is thoroughly homogenized. Use Zirconia Beads II and vortex for homogenization.
	Insufficient mixing of the sample with Binding Buffer A	Mix sample properly by pipetting before transferring the sample to the RTA Spin Filter membrane.
Low amount of nucleic acids from hard to lyse bacteria	Bacteria were not lysed during the lysis step at room temperature	After addition of Lysis Buffer P and vortexing, perform the following steps for sample lysis: Incubate 5 min at 90°C continuously shaking at 900 rpm Incubate 3 min on ice. Add 5 Zirconia Beads II. Incubate 3 min at 90°C continuously shaking at 900 rpm. Vortex the sample 2 min. Centrifuge for 1 min at 11.000 x g. Continue with the protocol step 2. Do not exceed incubation time at 90°C since the elevated temperature can increase the level of inhibitory compounds, like humic acid.
Degraded nucleic acids/ low A₂₆₀/A₂₈₀ ratio	Inefficient elimination of inhibitory substances due to insufficient mixing with the InviAdsorb matrix	Repeat the DNA purification procedure with a new sample, be sure to mix the sample and InviAdsorb matrix until the sample is thoroughly homogenized.
	Decreased Proteinase activity	Repeat the DNA purification procedure with a new sample and with Proteinase K. For difficult cases use double volume Proteinase K.
	Wash Buffer I and Wash Buffer II used in the wrong order	Ensure that Wash Buffer I and Wash Buffer II are used in the correct order in the protocol.

Nucleic acids do not perform well in downstream applications (e.g., real-time PCR or NGS)	Too much DNA used in downstream reaction	The extracted DNA can originate from many different organisms present in the original soil sample (e.g., animal, plant, bacteria). Reduce the amount of eluate or dilute the sample used in the downstream reaction.
	Sample lysis too long/too high temperature	Do not overextend heating steps which may lead to cleavage of humic acids, and by this to elevation of inhibitor contents.
	Copurification of inhibitors	See above.

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 Soil DNA Kit	50 preparations	1039100200
InviSorb® Spin Soil DNA Kit	250 preparations	1039100300

Revision history

Revision	Date	Description
V-01-2022	2022-07-04	New document
V-02-2022	2022-09-28	Revised temperature steps in short protocol
EN-v3-2023	2023-01-27	Protocol simplification, extension of troubleshoot section
EN-v4-2023	2023-04-19	Update contact details and corporate design to reflect the company's rebranding.
EN-v5-2024	2024-01-25	Update hazard and precautionary statements



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