Instructions for use InviSorb[®] Spin Food Kit















Invitek Molecular GmbH Robert-Rössle-Straße 10 Germany

Important notes

Thank you for purchasing the InviSorb® Spin Food Kit from Invitek Diagnostics.

The product serves the purpose of manual isolation of DNA from a wide range of food species of plant and animal origin (fresh, frozen or dried material from e.g. processed or unprocessed food containing meat, leaves, roots, fruits or seeds), 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 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: <u>www.invitek.com</u>

Contact: Technical support: <u>techsupport@invitek.com</u>

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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 <u>www.invitek.com</u>.

Dispose kit residues and waste fluids in accordance with your country's regulations, again refer to the MSDS. Invitek Molecular has not evaluated 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 Food Kit** to which they apply are listed below as follows:

Binding Buffer GT



Contains: Guanidiniumchlorid **Hazard statements** H302 - Harmful if swallowed. H315 - Causes skin irritation. H319 - Causes serious eye irritation. **Precautionary statements** P301+P312 - IF SWALLOWED: Call a POISON CENTRE or doctor if you feel unwell.

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

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



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 HL



Contains: Guanidiniumchlorid Hazard statements H302 - Harmful if swallowed. H315 - Causes skin irritation. H319 - Causes serious eye irritation. **Precautionary statements** P301+P312 - IF SWALLOWED: Call a POISON CENTRE or doctor if you feel unwell. P302+P352 - IF ON SKIN: Wash with plenty of water. P305+P351+P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P321 - Specific treatment (see supplemental first aid instruction on this label). P330 - Rinse mouth. P332+P313 - If skin irritation occurs: Get medical advice/attention. P337+P313 - If eye irritation persists: Get medical advice/attention. P362+P364 - Take off contaminated clothing and wash it before reuse. 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

InviSorb[®] Spin Food Kit

	50 purifications	
Catalogue No.	1036120200	
Lysis Buffer EM	1 x 60 ml/bottle	
Binding Buffer GT	1 x 15 ml/bottle	
Proteinase K	1 vial for 1.1 ml working solution	
RNase Free Water	1 x 15 ml/bottle	
Wash Buffer HL	1 x 15 ml/bottle (final volume 30 ml)	
Wash Buffer II	1 x 10 ml/bottle (final volume 50 ml)	
Elution Buffer M	1 x 15 ml/bottle	
RTA Spin Filter Set	1 x 50 sets	
RTA Receiver Tubes	1 x 50 pieces	
2.0 ml Safe-Lock Tubes	1 x 50 pieces	
1.5 ml Receiver Tubes	2 x 50 pieces	
Short Protocol	1 leaflet	

InviSorb[®] Spin Food Kit Add-On

	Content
Catalogue No.	1036020200
Lysis Buffer EM	2 x 60 ml/bottle
Proteinase K	2 vials for 2 x 1.1 ml working solution

2.2 Reagents and equipment to be supplied by user

Lab equipment:

- Microcentrifuge
- Thermo mixer (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)
- Optional: RNase A (10 mg/ml)

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 HL and II: after adding ethanol, 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.

2.4 Intended use.

The InviSorb[®] Spin Food 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 food material, DNA from pathogens such as bacteria may also be copurified.

The kit can be used for a wide range of food matrices of plant and animal origin (fresh, frozen or dried material from e.g., processed or unprocessed food containing meat, leaves, roots, fruits or seeds).

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 200 mg food sample	up to 50 µg (depending on sample type and volume)	A ₂₆₀ : A ₂₈₀ 1.6 –2.2	approx. 20 min (excl. lysis)

Yield and quality of purified nucleic acids depend on the sample type, sample source, transport, storage, and age. In addition, yield, and quality of isolated DNA from food samples depend on the type of food and especially on the degree of processing. Heavy processing of the food can cause a degradation of nucleic acids. Therefore, a lower DNA yield and fragmented DNA can be expected in this case.

If required, more than 200 mg of sample can be processed by linear upscaling of the lysis components. The additional Lysis Buffer EM and Proteinase K required for the upscaling can be purchased separately with the InviSorb[®] Spin Food Kit Add-On kit.

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 manufacturer's specifications.

2.6 Principle and procedure

1. Lyse samples

Before lysis, samples must be homogenized. Mortars, mills, or other commercial homogenizers (PowerLyzer, Bullet Blender etc.) can be used for homogenization. Samples are lysed under non-chaotropic conditions at elevated temperature. Lysis is performed with Lysis Buffer EM and Proteinase K. After sample lysis residues of food material are removed by centrifugation.

2. Bind DNA

By adding Binding Buffer GT and ethanol 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 HL and II, while the genomic DNA remains bound to the membrane.

4. Elute DNA

DNA is eluted from the Spin Filter using 100 μ l of prewarmed Elution Buffer. Optional the elution volume can be changed from 30-200 μ l to adjust DNA concentration of the eluate.

3. Nucleic acid extraction with the InviSorb[®] Spin Food 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 and reagent preparations prior first use: 50 preparations

Wash Buffer HL: Add 15 ml of 96 - 100% ethanol to the bottle. Mix thoroughly, always keep the bottle firmly closed.

Wash Buffer II: Add 40 ml of 96 - 100% ethanol to each bottle. Mix thoroughly, always keep the bottles firmly closed.

Proteinase K: Add 1,1 ml of RNase Free Water. Mix thoroughly by vortexing 10 sec.

- **Binding Buffer GT:** When processing many samples, a master mix of Binding Buffer GT and 96 100% ethanol can be prepared. For this purpose, Binding Buffer GT is mixed 1:1 with 96 100 % ethanol. 250 µl of Binding Buffer GT and 250 µl ethanol are required per sample.
- Adjust the thermo mixer to 65°C.
- Transfer the required amount of **Elution Buffer M** (100 µl **Elution Buffer M** 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 Filter RTA Sets, 2.0 ml Receiver Tubes and 1.5 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, storage, and degree of the food processing.

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

<u>Food material</u>: samples of food 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, freezing samples at -20° C or -80° C is recommended.

Dried sample material should be stored protected against humidity, sunlight and temperatures above 30°C.

3.3 **Preparation of starting materials**

In the following the preparation of the sample material is described.

For the extraction of DNA from food samples, it is important to perform a good homogenization to break up present structures and ensure that trace materials are evenly distributed. Mortars, mills, or other commercial homogenizers (PowerLyzer, Bullet Blender etc.) can be used for homogenization.

Homogenize a representative amount of sample and transfer up to 200 mg of homogenate into a 2.0 ml Safe-Lock Tube, to proceed with the lysis.

InviSorb® Spin Food Kit

3.4 Short protocol InviSorb[®] Spin Food Kit

Lyse samples

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

- 1. Transfer up to 200 mg of homogenized starting material to a 2.0 ml Safe-Lock Tube (provided).
- 2. Add 650 µl **Lysis Buffer EM** for non-dry material or 850 µl for dry material. Add 20 µl **Proteinase K**, vortex thoroughly for 15 sec.

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

Optional: To remove RNA from the sample, add 20 µl RNase A (10 mg/mL stock solution) per 200 mg of sample, vortex shortly and incubate for 30 min at room temperature.

- 3. Centrifuge at > 10,000 x g for 10 min to remove food residues.
- 4. Transfer 250 µl of the supernatant into a fresh 1.5 ml Receiver tube (provided).

Bind nucleic acids

- 5. Add 250 µl **Binding Buffer GT** and 250 µl **96 100 % ethanol** (or master mix), vortex for 30 sec.
- 6. Transfer the reaction mixture into an RTA Spin Filter Set and incubate for 1 min. Close the Spin Filter and centrifuge at 11.000 x g for 1 min. Discard the filtrate and place the Spin Filter back to the Receiver Tube.

Wash to remove residual contaminations

- 7. Add 400 μl **Wash Buffer HL**, 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 700 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 into a new RTA Receiver Tube.
- Add 200 µl Wash Buffer II, close the Spin Filter and centrifuge at 11.000 x g for 3 min to remove residual Wash Buffer II. Carefully remove the filter from the Receiver Tube (without touching the Wash Buffer II in the tube).

Elute nucleic acids

10. Place the Spin Filter in a new 1.5 ml Receiver Tube and add 100 μl **Elution Buffer M** (preheated to 65°C) directly onto the Spin Filter. Incubate for 5 min at RT. Centrifuge for 1 min at 11.000 x g. Discard the Spin Filter and store eluted nucleic acids on ice.

The eluate can be stored at 4 $^\circ\text{C}$ up to 3 days or at –20 $^\circ\text{C}$ for longer periods.





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

Please refer to chapter 3.3 "Preparation of starting material" for sample specific pretreatment.

Please follow your local regulatory SOPs for food analysis. The lysis process described in this protocol is optimised for 200 mg sample. If you need to process and analyse more starting material or difficult samples, we recommend linear upscaling of the components for lysis (Lysis Buffer EM and Proteinase K). Additional Lysis Buffer EM and Proteinase K can be ordered separately as an add-on (InviSorb[®] Spin Food Kit Add-On; Catalogue No. 1036020200).

- 1. Transfer up to 200 mg sample to a 2.0 ml Safe-Lock Tube (provided).
- Add 650 µL Lysis Buffer EM for non-dry material or 850 µl for dry material. Add 20 µL Proteinase K and vortex thoroughly for 15 sec. If the sample is not fully suspended, add more Lysis Buffer EM and Proteinase K until it is suspended. Incubate the reaction mix at 65°C for 30 min or longer vigorously shaking (800 rpm or more). Prolonged incubation time in the lysis buffer obtains higher DNA yields.

<u>Optional</u>: To remove RNA from the sample, after lysis, add 20 µl RNase A (10 mg/ml stock solution) per 200 mg of sample, vortex shortly and incubate for 30 min at room temperature.

- 3. Centrifuge at > 10,000 x g for 10 min to remove food residues.
- 4. Transfer 250 µl of the supernatant into a fresh 1.5 ml Receiver tube (provided).

<u>Note</u>: According to the nature of the starting material, solid food residues are at the bottom of the tube, oils, and fatty acids on top of the aqueous phase. Remove the aqueous phase without disturbing the pellet or the oily phase (minor oil residues are removed in the following washing steps).

5. Add 250 µl **Binding Buffer GT** and 250 µl **ethanol** (96 - 100 %) to the supernatant, vortex for 30 sec.

Optional: When processing many samples, a master mix of Binding Buffer GT and 96-100% ethanol can be prepared by mixing the two components 1:1.

- Transfer the reaction mixture into an RTA Spin Filter Set and incubate for one minute. Close the Spin Filter and centrifuge at 11.000 x g for 1 min. Discard the filtrate and place the Spin Filter back to the Receiver Tube.
- 7. Add 400 μl **Wash Buffer HL**, close the Spin Filter and centrifuge at 11.000 x g for 1 min. Discard the filtrate, place the Spin Filter back into the Receiver Tube.
- 8. Add 700 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 into a new RTA Receiver Tube.
- Add 200 µl Wash Buffer II, close the Spin Filter and centrifuge at 11.000 x g for 3 min to remove residual ethanol. Carefully remove the Spin Filter r from the Receiver Tube. Avoid touching the Wash Buffer II in the tube.
- 10. Place the Spin Filter in a new 1.5 ml Receiver Tube and add 100 μl **Elution Buffer M** (preheated to 65°C) directly onto the Spin Filter. Incubate for 5 min at room temperature. Centrifuge for 1 min at 11.000 x g. Discard the Spin Filter and store eluted nucleic acids on ice.

The eluate can be stored at 4 $^{\circ}$ C up to 3 days or at -20 $^{\circ}$ C for longer periods.

4. Appendix

4.1 Troubleshooting

Problem	Possible cause	Recommendation
Low amount of nucleic acids	Insufficient cell lysis	Increase lysis time with Lysis Buffer EM and Proteinase K. Continuous shaking improves lysis efficiency. Reduce amount of starting material to avoid column overload.
	Incomplete elution	Increase incubation time with preheated Elution Buffer M to 5-10 min. Elute twice with the same volume of Elution Buffer M. Use a higher volume of Elution Buffer M (max. 200 µl).
	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.
	Too much Elution Buffer	Elute with a lower volume of Elution Buffer (min. 30 µl).
	Old material	Ensure that the starting material is stored at appropriate conditions (–20°C/–80°C).
	Starting material contains low amount of nucleic acids	Scale up Lysate-Binding-Mixture (e.g., 350 µl Lysate + 350 µl Binding Buffer GT + 350 µl ethanol (96 - 100 %)). Load sequentially onto the spin filter.
Degraded nucleic acids	Incorrect storage of starting material	Ensure that the starting material is stored at appropriate conditions (–20°C/–80°C).
	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.
DNA does not perform well in	Ethanol carryover during elution	Increase time of drying step for removal of ethanol.
applications (e.g., real-time PCR or NGS)	Insufficient cell lysis and/or too much starting material	See above remark about insufficient cell lysis. Increase centrifugation time/speed. Reduce the amount of starting material.
Clogged Spin Filter	High level of residual RNA or proteins	Perform RNase A treatment as described in the protocol and prolong the lysis time

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 assessed 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 <u>www.invitek.com</u>. If you have any questions, please contact <u>techsupport@invitek.com</u>.

4.3 Symbols used on product and labelling.

	Manufacturer
LOT	Lot number
REF	Catalogue number
2	Expiry date
ī	Consult operating instructions
	Temperature limitation
\otimes	Do not reuse
Σ	Amount of sample preparations

4.4 Further documents and supplementary information

Visit <u>www.invitek.com</u> 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 <u>techsupport@invitek.com</u> or the dealer responsible for you.

4.5 Ordering information

Product	Package Size	Catalogue No
InviSorb [®] Spin Food Kit	50 preparations	1036120200
InviSorb [®] Spin Food Kit Add-On	120 ml	1036020200

Revision history

Revision	Date	Description
EN-v1-2024	2024-03-01	New document

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