



# USER MANUAL

## Invisorb<sup>®</sup> Spin Plant Mini Kit

for genomic DNA purification from up to 100 mg wet and up to 60 mg of dried plant material from a wide variety of plant species as well as from food samples of plant origin

## Instruction for the Invisorb® Spin Plant Mini Kit

The **Invisorb® Spin Plant Mini Kit** has been designed for isolation and purification of total DNA from a wide variety of plant species (fresh, frozen or dried plant material, for instance leaves, roots, fruits or seeds) and also from a wide variety of food sample from plant origin (fresh, frozen or dried material).

The DNA extraction and purification chemistry was intensely tested and validated.

The **Invisorb® Spin Plant Mini Kit** is intended for life science research use only. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations.

The kit is neither suitable for isolation of RNA from plant material and food sample from plant origin, as for DNA isolation from cultured or isolated cells, tissue samples or blood samples. Simultaneously isolation of bacterial DNA or DNA from fungi and parasites is not validated.

Trademarks: Invisorb®. Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110,363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

Invisorb® is a registered trademark of Invitex Molecular GmbH.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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## Kit contents of the Invisorb® Spin Plant Mini Kit

	250 DNA preps
Catalogue No.	1037100300
Lysis Buffer P	120 ml
Binding Buffer A	2 x 9 ml (final volume 2 x 30 ml)
Proteinase S	3 x 2 ml
Wash Buffer I	80 ml (final volume 160 ml)
Wash Buffer II	2 x 45 ml (final volume 2 x 150 ml)
Elution Buffer	60 ml
Prefilter	5 x 50
Spin Filter	5 x 50
2.0 ml Receiver Tubes	10 x 50
1.5 ml Receiver Tubes	5 x 50
Manual	1
Initial steps	<p>Add 21 ml 99.7% Isopropanol to each <b>Binding Buffer A</b>. Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.</p> <p>Add 80 ml of 96-100% Ethanol to the bottle <b>Wash Buffer I</b>.</p> <p>Add 105 ml of 96-100% Ethanol to each bottle <b>Wash Buffer II</b>, mix thoroughly and store with tightly closed cap.</p> <p>Incubate the needed amount of <b>Elution Buffer</b> at 65°C in a Thermomixer.</p>

## Symbols

	Manufacturer
	Lot number
	Catalogue number
	Expiry date
	Consult operating instructions
	Temperature limitation
	Do not reuse
	Humidity limitation

**Attention:** Do not combine components of different kits, unless the lot numbers are identical!

## Storage

All buffers and kit components of the **Invisorb® Spin Plant Mini Kit** should be stored at room temperature (RT) and are stable for 12 months under these conditions.

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

Before every use, make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by warming carefully (up to 30°C).

**Wash Buffer** charged with ethanol should be appropriately sealed and stored at room temperature.

**Binding Buffer A** charged with isopropanol should be appropriately sealed and stored at room temperature.

## Quality control and product warranty

Invitek Molecular warrants the correct function of the **Invisorb® Spin Plant Mini Kit** for applications as described in this manual. Purchaser must determine the suitability of the Product for its particular use. Should any Product fail to perform the applications as described in the manual, Invitek Molecular will check the lot and if Invitek Molecular investigates a problem in the lot, Invitek Molecular will replace the Product free of charge.

Invitek Molecular reserves the right to change, alter, or modify any Product to enhance its performance and design at any time.

In accordance with Invitek Molecular's EN ISO 13485 certified Quality Management System the performance of all components of the **Invisorb® Spin Plant Mini Kit** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **Invisorb® Spin Plant Mini Kit** or other Invitek Molecular products, please do not hesitate to contact us. A copy of Invitek Molecular's terms and conditions can be obtained upon request or are presented at the Invitek Molecular webpage ([www.invitek-molecular.com](http://www.invitek-molecular.com)).

**For technical support or further information please contact:**

**from Germany**

**+49-(0)30-9489-2901/ 2910**

**from abroad**

**+49-(0)30-9489-2907**

**or contact your local distributor.**

## Intended use

The **Invisorb® Spin Plant Mini Kit** is the ideal tools for a rapid and efficient isolation of high quality genomic DNA from up to 100 mg of a wide variety of plant species (fresh, frozen or dried plant material, for instance leaves, roots, fruits or seeds) and from food sample from plant origin.

The protocols for the isolation and all buffers are optimized for a high yield as well as a high purity. All hands on steps are reduced to a minimum. For reproducible and high yields, appropriate sample storage is essential.

THE PRODUCT IS INTENDED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modification of DNA followed by signal detection or amplification. Any results generated using the sample preparation procedure in conjunction with any downstream assay should be interpreted with regard to other laboratory findings.

To minimize irregularities in your results, adequate controls for downstream applications should be used.

## Product use limitation

The kit is neither suitable for isolation of RNA from plant material, as for DNA isolation from cultured or isolated cells, tissue samples or blood samples. The isolation of simultaneously isolation of bacterial DNA or DNA from fungi and parasites is not validated.

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore, neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the Invitek Molecular Product for any particular use. Invitek Molecular does not provide for validation of performance characteristics of the Product with respect to specific applications.

Invitek Molecular products may be used e.g. in clinical diagnostic laboratory systems under following conditions:

- If used in the US, based on the condition that the complete diagnostic system of the laboratory has been validated pursuant to CLIA' 88 regulations.
- For other countries based on the condition that the laboratory has been validated pursuant to equivalents according to the respective legal basis.

All Products sold by Invitek Molecular are subject to extensive quality control procedures (according to EN ISO 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to Invitek Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The Product with its contents is unfit for consumption.

## Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at [www.invitek-molecular.com](http://www.invitek-molecular.com) for each Invitek Molecular Product and its components. If buffer bottles are damaged or leaking, WEAR GLOVES, AND PROTECTIVE GOGGLES when discarding the bottles in order to avoid any injuries.

Invitek Molecular has not tested the liquid waste generated by the **Invisorb® Spin Plant Mini Kit** procedure for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

European Community risk and safety phrases for the components of the **Invisorb® Spin Plant Mini Kit** to which they apply, are listed below as follows:

### Proteinase S:



Danger

H317-H318-P280-P305+P351+P338

### Lysis Buffer P



Warning

H319-H412-P280-P305+P351+P338-P273

### Wash Buffer I



Warning

H302-412-P280-P305-P351-P338-P273-EUH032

H302: Harmful if swallowed.

H317: May cause an allergic skin reaction.

H318: Causes serious eye damage.

H319: Causes serious eye irritation.

P273: Avoid release to the environment.

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.

H412: Harmful to aquatic life with long lasting effects.

EUH032: Contact with acids liberates very toxic gas.

### Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: 1 – 352 – 323 – 3500

in USA : 1 – 800 – 535 – 5053

## Product characteristic of the Invisorb® Spin Plant Mini Kit

Starting material	Yield	Time for preparation	Ratio
up to 100 mg of plant material up to 60 mg of dried plant material	up to 50 µg depends on amount and kind of starting material	about 20 minutes after lysis	$A_{260} : A_{280}$ 1.6 – 2.0

The **Invisorb® Spin Plant Mini Kit** provide a very efficient procedure for isolation of high quality genomic DNA from a wide variety of plant species

The **Invisorb® Spin Plant Mini Kit** combine the lysis of starting material with the very efficient binding of genomic DNA onto a spin filter surface without chaotropic ions.

The isolation protocol as well as all buffers is optimized to provide high yield and purity of the isolated genomic DNA. The “hands-on time“ necessary for the whole procedure is reduced to minimum.

The purification procedure is rapid and requires neither phenol/ chloroform extraction nor alcohol precipitation, and requires minimal interaction by the user.

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

### Downstream Application:

- PCR \*)
- RFLP-Analysis
- Restriction Enzyme Digestion
- Southern Blot Analysis
- Sequencing
- Cloning

**For further information please contact:** Tel.: +49 (0) 30 9489 2901, 2910 in Germany and from foreign countries Tel.: +49 (0) 30 9489 2907 or your local distributor.

\*) The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.



## Principle and procedure

The **Invisorb® Spin Plant Mini Kit** procedure comprises following steps:

- lysis of sample material
- binding the genomic DNA to the membrane
- washing and elimination of ethanol
- elution of genomic DNA

**Proteinase S** is colored blue; this is for tracking the correct addition of the small volume enzyme.

After lysis the DNA binds to the membrane, contaminations and enzyme inhibitors are efficiently removed during the following wash steps and highly purified DNA is eluted in Elution Buffer or water.

## Sampling and storage of starting material

Harvested plant samples/ food sample 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. Multiple thawing and freezing before isolating the DNA should be avoided.

## Important notes

### Important points before starting a protocol

Immediately upon receipt of the Product, inspect the Product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify Invitex Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the Invitex Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 7). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps are carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Discard gloves if they become contaminated.
- Do not combine components of different kits unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by trained personnel.

## Important indications

1. Process only as much plant samples as the microcentrifuge allows to process.
2. Plant sample and buffers should be thoroughly mixed and should have room temperature
3. The elution can be done by using lower amount of **Elution Buffer**. This may result in a higher concentration of DNA. However, pay attention that minimum volume for elution is 50 µl, but this will reduce the yield.
4. The eluted DNA volume can be lower than the added **Elution Buffer** volume.
5. **Elution Buffer** should be preheated to 65 °C.
6. The **Elution Buffer** does not contain EDTA.
7. The yield can be increased, if the incubation time with preheated **Elution Buffer** will be prolonged.

## Yield and quality of genomic DNA

The amount of purified DNA in the **Invisorb® Spin Plant Mini Kit** procedure from plant material depends on sample source, transport conditions, storage and age of the sample. Yield and quality of isolated genomic DNA is suitable for any detection system.

## Preparing reagents and buffers of the Invisorb® Spin Plant Mini Kit

1. Adjust the thermo mixer to 65°C.
2. Warm up the needed amount of **Elution Buffer** to 65°C.
3. Label the needed amount of Spin Filter.
4. Label the needed amount of reaction tubes.
5. Add the needed amount of ethanol to the **Wash Buffers**.

## Reagents and equipment to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at [www.invitek-molecular.com](http://www.invitek-molecular.com) under each Invitek Molecular kit and kit component.

- Microcentrifuge
- Thermomixer (for 65°C)
- Reaction tubes (1.5 ml)
- dd H<sub>2</sub>O
- 96 - 100 % ethanol
- RNase A (10 mg/ ml - optional)
- Isopropanol\*

\*The **Invisorb® Spin Plant Mini 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

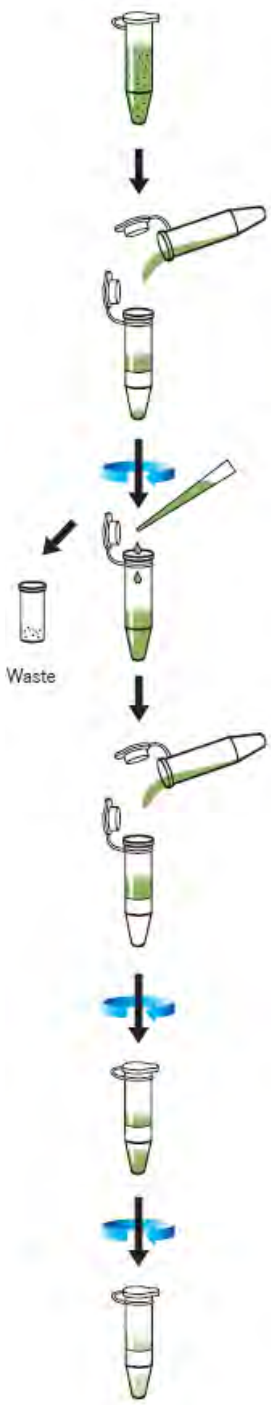
#### **Sigma**

2-Propanol  
Order no. 59304-1L-F

### 250 DNA extractions:

Add 21 ml 99.7% Isopropanol to each **Binding Buffer A**.  
Add 80 ml 96-100% Ethanol to the bottle **Wash Buffer I**.  
Add 105 ml 96-100% Ethanol to each bottle **Wash Buffer II**, mix thoroughly and store with tightly closed cap.

## Scheme of the Invisorb® Spin Plant Mini Kit

 <p><b>genomic DNA</b></p>	<p><b>Please read protocols prior the start of the preparation carefully</b></p> <hr/> <p>Homogenize about 60 mg of starting material by a pestle under liquid N<sub>2</sub></p> <p>Transfer homogenized plant material into a 1.5 ml reaction tube. Add 400 µl <b>Lysis Buffer P</b> and 20 µl <b>Proteinase S</b>, vortex briefly. Incubation at 65°C for 30 min</p> <p>Transfer of Lysis Solution onto the Prefilter. Centrifuge for 1 min at 11.100 x g (11.000 rpm) Discard the Prefilter</p> <p>Add 200 µl of <b>Binding Buffer A</b> (<i>follow preparing instructions</i>) and vortex thoroughly</p> <p>Transfer the suspension onto the Spin Filter. Incubate for 1 min. Centrifuge at 11.100 x g (11.000 rpm) for 2 min. Discard the filtrate</p> <p>Add 550 µl <b>Wash Buffer I</b>. Centrifuge at 11.100 x g (11.000 rpm) for 1min. Discard the filtrate Place the Spin Filter again into the 2.0 ml Receiver Tube.</p> <p>Add 550 µl <b>Wash Buffer II</b> and centrifuge at 11.100 x g (11.000 rpm) for 1min. Discard the filtrate, Repeat the washing step once again. Discard the filtrate and centrifuge for 4 min at 11.100 x g (11.000 rpm)</p> <p>Place the Spin Filter into a new 1.5 ml Receiver Tube and add 100 µl of the prewarmed <b>Elution Buffer</b>. Incubate for 3 min. Centrifuge for 1 min at 11.100 x g (11.000 rpm).</p> <p>Discard Spin Filter Close the 1.5 ml Receiver Tube and store the DNA sample at 4 °C</p>
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## **Protocol: DNA extraction from fresh or dried plant material and from food of plant origin (fresh, frozen or dried material)**

Please read the instructions carefully and conduct the prepared procedure.

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**Attention:** Please be aware, that you have to prepare the **Binding Buffer A** – see instruction page: 9

**Important** Transfer the needed amount of **Elution Buffer** into 2.0 ml Receiver Tube (not included in the kit) and place the tube a 65°C.

### **1. Homogenization of the starting material**

Homogenize about 60 mg of starting material by a pestle under liquid N<sub>2</sub>. Commercially available equipment for homogenization also can be used.

**Note:** Use 120-180 mg of starting material if extraction from material containing more water (fruits, algae).

### **2. Lysis of the starting material**

Transfer the “plant powder” into a 1.5 ml reaction tube. Add 400 µl **Lysis Buffer P** and 20 µl **Proteinase S** and vortex briefly. Incubation at 65°C for 30 min or longer (incubation in a thermomixer under continuous shaking is recommended). During incubation, place the Prefilter into a 2.0 ml Receiver Tube.

**Option:** Transfer the “plant powder” into a 1.5 ml reaction tube. Add 400 µl **Lysis Buffer P** and 20 µl **Lysozyme** and vortex briefly. Incubation at 37°C for 10 min. Add 20 µl **Proteinase S** and vortex briefly. Incubation at 65°C for 30 min or longer (incubation in a thermo mixer under continuous shaking is recommended). During incubation, place the Prefilter into a 2.0 ml Receiver Tube.

### **3. Filtration of Lysis Solution and realizing optimum binding conditions**

Transfer the Lysis Solution onto the Prefilter. Centrifuge for 1 min at 11.100 x g (11.000 rpm) Discard the Prefilter.

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

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

### **4. DNA Binding**

Place the Spin Filter into a 2.0 ml Receiver Tube. Transfer the suspension onto the Spin Filter and incubate for 1 min. Centrifuge at 11.100 x g (11.000 rpm) for 2 min. Discard the filtrate and place the Spin Filter again into the 2.0 ml Receiver Tube.

### **5. Washing I**

Add 550 µl **Wash Buffer I** and centrifuge at 11.100 x g (11.000 rpm) for 1min. Discard the filtrate, place the Spin Filter again into the 2.0 ml Receiver Tube.

### **6. Washing II**

Add 550 µl **Wash Buffer II** and centrifuge at 11.100 x g (11.000 rpm) for 1min. Discard the filtrate, place the Spin Filter again into the Receiver Tube and repeat the washing step once again. Finally discard the filtrate and centrifuge for 4 min at 11.100 x g (11.000 rpm) to remove residual ethanol.

### **7. Elution of the DNA**

Place the Spin Filter into a new 1.5 ml Receiver Tube and add 100 µl of the prewarmed **Elution Buffer**. Incubate for 3 min. Centrifuge for 1 min at 11.100 x g (11.000 rpm).

**Note:** The DNA can also be eluted with a lower or a higher volume of Elution Buffer (depends on the expected yield of genomic DNA). However, pay attention, that minimum volume for the elution is 50 µl. If quite large amount of DNA is expected, the volume of elution can be increased (100-200 µl).  
**To maximize the final yield we recommend a second elution step with the equal volume of Elution Buffer.**

## Troubleshooting

Problem	Cause	Comments and suggestions
<b>clogged Spin Filter</b>	insufficient lysis and/or too much starting material	increase lysis time increase centrifugation speed or time reduce amount of starting material
<b>low amount of extracted DNA</b>	insufficient lysis  incomplete elution  insufficient mixing with <b>Binding Buffer A</b>	increase lysis time reduce amount of starting material overloading of Spin Filter reduces yield prolong the incubation time with <b>Elution Buffer</b> to 5-10 min or repeat elution step once again  take higher volume of <b>Elution Buffer</b> Mix sample with <b>Binding Buffer A</b> by pipetting or by vortexing prior to transfer the sample onto the Spin Filter
<b>low concentration of extracted DNA</b>	too much <b>Elution Buffer</b>	elute the DNA with lower volume of <b>Elution Buffer</b>
<b>degraded or sheared DNA</b>	incorrect storage of starting material  old material	ensure that the starting material is frozen immediately in liquid N <sub>2</sub> or in minimum at -20°C and is stored continuously at -80°C avoid thawing and freezing of the material old material often contains degraded DNA
<b>RNA contaminations of extracted DNA</b>	to much RNA	RNAse A digestion
<b>genomic DNA does not perform well in downstream-applications</b>	ethanol carryover during elution  Salt carryover during elution	increase time for removing of ethanol ensure that <b>Wash Buffer</b> is at room temperature  check up <b>Wash Buffer</b> for salt precipitates. If there are any precipitates, solve these precipitates by careful warming

## **Appendix**

### **General notes on handling DNA**

#### **Nature of DNA**

The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting and long-template PCR.

#### **Storage of DNA**

A working stock of DNA can be stored at 2 – 4°C for several weeks. For long term storage DNA should be stored at -20°C, but storing at – 20°C can cause shearing, particularly if the DNA is exposed to repeated freeze-thaw cycles.

Note that the solution in which the nucleic acid is eluted in will affect its stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

#### **Drying, dissolving and pipetting DNA**

Avoid over drying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution.

Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

#### **DNA Yield**

The amount of purified DNA from the plant material depends on sample source, transport conditions, storage and age of the sample.

## Ordering information

<b>Product</b>	<b>Package size</b>	<b>Catalogue No.</b>
Invisorb® Spin Plant Mini Kit	250 preparations	1037100300

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### Related products

Invisorb® DNA Plant 96 HTS/ C	4 x 96 preparations	7037300300
Invisorb® DNA Plant 96 HTS/ C	24 x 96 preparations	7037300400

### 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

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