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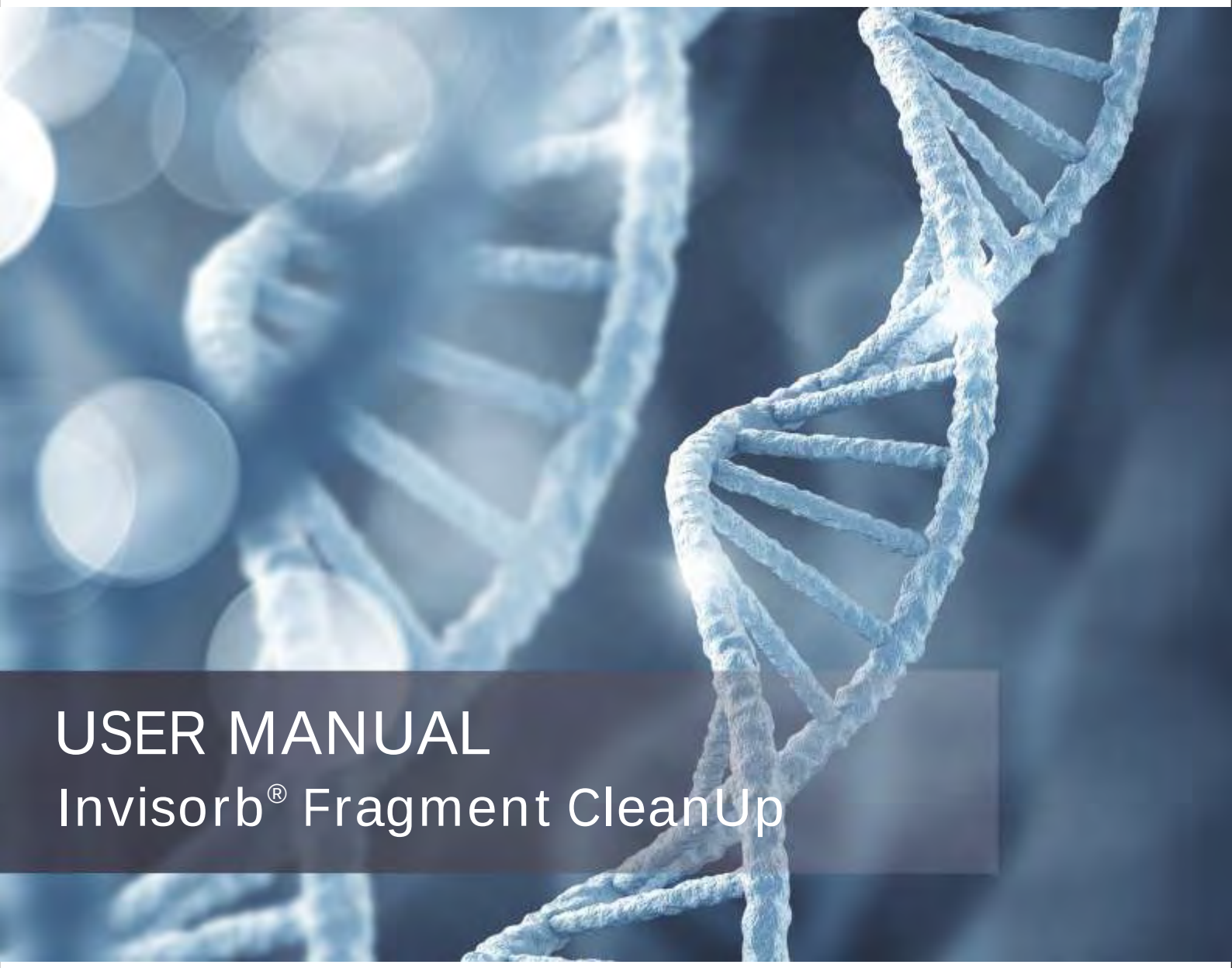


USER MANUAL
Invisorb® Fragment CleanUp

for purification of DNA fragments after PCR and other enzymatic reactions and
for extraction of DNA fragments from agarose gels

REF 1020300300

Invitek Molecular GmbH · D-13125 Berlin

Instruction

Invisorb® Fragment CleanUp

The **Invisorb® Fragment CleanUp** provides a convenient tool for fast and efficient purification of PCR products and DNA fragments from amplification or enzymatic reactions.
Furthermore, this kit is the ideal tool for extraction of DNA fragments of 80 bp – 30 kb from standard or low melting agarose gels in TAE and TBE buffers at high final DNA concentrations. Up to 300 mg agarose gel slices can be processed per spin column.

Contents

Kit contents of Invisorb® Fragment CleanUp

	250 preps
Catalogue No.	1020300300
Gel Solubilizer S	2 x 140 ml
Binding Buffer	63 ml (final volume 163 ml)
Binding Enhancer	30 ml (final volume 150 ml)
Wash Buffer	2 x 45 ml (final volume 2 x 150) ml)
Elution Buffer	15 ml
Spin Filter	5 x 50
2.0 ml Receiver Tubes	5 x 50
1.5 ml Receiver Tubes	5 x 50
Manual	1
Initial steps	add 105 ml 96-100% ethanol to each bottle **Wash Buffer** add 120 ml 99.7% Isopropanol to the **Binding Enhancer;** Mix by intensive shaking for 1 min. Shortly before use mix by inverting several times add 100 ml 99.7% Isopropanol to the **Binding Buffer;** Mix by intensive shaking for 1 min. Shortly before use mix by inverting several times

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 contents of the **Invisorb® Fragment CleanUp** should be stored at room temperature and are stable for at least 12 months.

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

Before every use make sure that all components have room temperature. If there are any precipitates in the provided solutions, they can be dissolved by careful warming (up to 30°C).

Quality control and product warranty

Invitek Molecular guarantees the full functionality of the **Invisorb® Fragment CleanUp** for applications described in this manual. The user must examine the suitability of the product for its particular use. If a product does not meet the requirements for an application described in this manual, Invitek Molecular will check the lot number of the product. If a problem is identified by the manufacturer, the product will be replaced 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® Fragment CleanUp** have been tested separately against predetermined specifications.

If you have any questions or problems regarding any aspects of the **Invisorb® Fragment CleanUp** 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 is available at the Invitek Molecular webpage www.invitek-molecular.com.

For technical support or further information, please contact:
Email: techsupport@invitek-molecular.com or contact your local distributor.

Intended use

Before using the kit, please ensure that you have read the instructions and are fully informed about the purpose and limitations of use of the kit (see "Product use limitation", and "Features of the Invisorb® Fragment CleanUp").

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 modifications of DNA followed by signal detection or amplification.

The kit is developed, designed, and sold for research purposes only. They are neither to be used for human diagnostic nor to be administered to humans unless explicitly cleared for that purpose by the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of the materials described in this text.

Product use limitation

For purification DNA fragments should not be bigger than 30 kb and not smaller than 80 bp. The maximum length of primers which can be removed is 40 bp. The Invisorb® Fragment CleanUp is not suitable for extraction of circular plasmids as these can be detected in multiple positions in the gel due to their very different configurations.
When carrying out the PCR with Taq DNA Polymerase, it is possible that the "A-overhangs" may be lost during extraction. It is therefore recommended to repair the A-overhangs if they are needed in the subsequent downstream application.
The included chemicals can only be used once. Any deviation of the source material or the flow line can lead to inoperability; therefore, in this case, no warranty or guarantee is given, either implicitly or explicitly. Invitek Molecular will be released of its responsibilities if other sample materials than described are processed or if the sample preparation protocols are changed or modified.
The user is responsible to validate the performance of the Invitek Molecular product for any particular use. Invitek Molecular does not provide a validation of the performance characteristics of the product in relation 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 according 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 it is guaranteed that they work as described here. Any problems, incidents or defects shall be reported to Invitek Molecular immediately upon detection.
The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for other purposes than intended. The product is not suitable for consumption.

Safety information

When and while working with chemicals, always wear a lab coat, disposable gloves, and protective goggles! Avoid skin contact! Adhere to the legal requirements for working with biological materials! 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 for all Invitek Molecular products and their 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 the **Invisorb® Fragment CleanUp** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is very unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and must be handled and discarded according to local safety regulations.
European Community risk and safety phrases for the components of the **Invisorb® Fragment CleanUp** are listed as follows:

Gel Solubilizer

Warning

H302-312-332-412--P280-P305+P351+P338 -EUH032

H302: Harmful if swallowed.
H312: Harmful in contact with skin.
H332: Harmful if inhaled.
H412: Harmful to aquatic life with long lasting effects.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
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

PCR product & DNA fragment purification and concentration

This manual describes one kit for both, the membrane adsorption-based purification via high-performance MSB® technology of PCR products and products from other enzymatic reactions as well as the purification of DNA fragments from agarose gels using the **Invisorb®** spin columns via the standard lyse-bind-wash-elute-protocol.

	Gel Solubilization Technology
Sample Volume	up to 100 µl * or up to 300 mg gel slices
Recovery	80 – 95 %* 60 – 90 %
Binding capacity	10 µg
Elution Volume (minimal)	10 µl */ 20 µl
Sample Source :	
- PCR reaction mixture	x*
- Ligation reaction mixture	x*
- Enzyme digestion mixture	x*
- cDNA synthesis mixture	x*
- Cycle sequencing reaction	x*
- DNA fragments	x*
- Agarose gels (TAE, TBE)	x

* *MSB® technology*

Advantages

- convenient and fast sample processing
- the most efficient removal of contaminants
- high recovery rate of PCR products or DNA fragments
- broad range of fragment sizes: 80 bp - 30 kb can be purified

The spin columns are designed to give high final concentrations of purified DNA fragments for subsequent reactions. Special buffers provided with this kit are optimized for efficient recovery of DNA and removal of contaminants like, salts, enzymes, nucleotides, agarose, ethidium bromide and other impurities from DNA samples. Specialized binding buffers promote selective adsorption of DNA fragments and PCR products. The pure DNA is eluted in a small volume of buffer or water, ready to use for any subsequent application. The innovative **MSB® technology** is described in the following chapter.

MSB® technology

The fastest technology for purification of DNA fragments with high recovery rates.

The MSB® technology offers a new option for the purification process in which handling steps are greatly simplified and processing times are significantly reduced. The products with **MSB® technology** have been designed for extremely efficient purification and/or concentration of PCR products and DNA fragments from enzymatic reaction mixtures in **only two steps**.

The DNA fragments adsorb at the silica membrane in the presence of minimal concentrations of non-chaotropic salts, while impurities pass through the column. Therefore, a washing step is not required. High concentrated, pure DNA fragments are eluted ready for use.

Advantages:

- ultra-fast and easy (two step protocol), only binding and elution
- excellent purity without washing
- 80 – 95 % recovery rate

DNA purified with the MSB® technology is much more concentrated than DNA purified by other methods. The highly concentrated DNA allows the use of small reaction volumes, which are useful for any downstream application, leading to increased efficiency (e.g. in ligations).

Features of the Invisorb® Fragment CleanUp

Starting material **Size of DNA fragments: 80 bp - 30 kb**	**Yield**	Time for preparation
up to 100 µl reaction volume like PCR reaction mixture, up to 100 µl restriction digestion mixture, up to 100 µl ligation mixture, up to 100 µl cDNA synthesis mixture, up to 100 µl cycle sequencing reaction mixture	80 – 95 %, depends on fragment length	approx. 7 min
up to 300 mg of gel slices (0.8 – 2%) from TAE or TBE agarose gels	60 - 90 %, depends on fragment length and kind of agarose gel	approx. 20 min

The **Invisorb® Fragment CleanUp** provides ultra-fast purification and concentration of up to 100 µl PCR products, or of linearized plasmid DNA as well as purification of DNA fragments from agarose gels and clean-up of other enzymatic reactions.

For PCR purification the MSB® method is used, which requires no washing steps, DNA fragments are eluted with low salt buffer or water.

For purification of DNA-fragments from agarose gels, the standard lyse-bind-wash-elute-protocol is used. DNA fragments bind directly to the surface of a spin filter column after gel solubilization. After washing steps, elution of the DNA fragments is performed with a low salt buffer.

While the MSB® method works without chaotropic salts, chaotropic salts are necessary for lysis from an agarose gel. Therefore, depending on the protocol, both types of chemistry, chaotropic or non-chaotropic, are used for DNA binding in this kit.

The purified DNA-fragments are ready to use in various downstream applications such as:

- digestion with restriction enzymes
- hybridization, labelling, cloning
- sequencing
- *In vitro* transcription
- ligation and transformation
- DNA sequencing
- amplification

Important notes

Important points before starting a protocol

Check the product and its components as well as the packaging for obvious damage, correct quantities and quality immediately upon receipt of the product. If there are any deviations, please notify Invitek Molecular in writing with immediate effect after examination. If bottles containing buffer are damaged, contact the Invitek Molecular Technical Services or your local distributor. In case of liquid spillage, refer to the “Safety Information” (see page 5). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipette tips between liquid transfers. To avoid cross-contamination the use of filter tips is recommend.
- All centrifugation steps are carried out at room temperature.
- When working with chemicals, always wear a lab coat, disposable gloves and protective goggles.
- Discard gloves if they become contaminated.
- Do not mix kit components with components from other 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 personnel trained in vitro diagnostic laboratory practice.

Equipment and reagents to be provided by the user

- Microcentrifuge (≥ 11.100 x g)
- Ethanol (96-100%)
- Thermoshaker
- Pipettes and filter tips
- Scalpel 120
- 1.5 ml and 2.0 ml reaction rubes
- Isopropanol (99.7%)

*The **Invisorb® Fragment CleanUp** 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 ≥99.8%, p.a., ACS, ISO
Ordering No. 6752

Applichem
2-Propanol für die Molekularbiologie
Ordering No. A3928

Sigma
2-Propanol
Ordering No. 59304-1L-F

Principle and procedure of the Invisorb® Fragment CleanUp

The **Invisorb® Fragment CleanUp** combines two applications: DNA fragment purification and agarose gel extraction.

Procedure for DNA fragment purification:

- selective binding of DNA fragments to the surface of the DNA-Binding Spin Filter
- elimination of enzyme buffer, enzyme, primers and nucleotides during the binding step
- elution of the highly pure DNA fragment

Sampling and storage of starting material

Best results are obtained using freshly prepared PCR or enzymatic reaction mixtures to prevent DNA digestion. The samples can be stored for some weeks at 4 - 8°C.

Binding of DNA fragments

The reaction mixture is mixed in a ratio of 1:5 with the **Binding Buffer** to provide the appropriate condition for the binding of DNA fragments in a range of 80 bp - 30 kb to the silica membrane under minimal concentrations of non-chaotropic salts.
The binding of small DNA fragments can be supported by the addition of small amounts of isopropanol, but this ratio is very sensitive.

Removal of Contaminants

The DNA fragments bind to the membrane at minimal concentrations of non-chaotropic salts. Therefore, a washing step is not required. Unwanted primers and impurities such as salts, enzymes, unincorporated nucleotides, dyes, ethidium bromide, oils, and detergents do not bind to the silica membrane; instead, they are pulled through the column by centrifugal force together with the large access of Binding Buffer. Any remaining Binding Buffer, which may interfere with subsequent enzymatic reactions, is removed by additional centrifugation.

Elution of PCR products or DNA fragments

DNA is eluted from the column using 10 - 50 µl Elution Buffer.
Eluting twice each with 30 - 50 µl leads to complete recovery of DNA. By the use of smaller elution volumes DNA concentration can be increased. Elution volumes should not fall below 10 µl, otherwise the yield will be reduced. The eluted DNA is ready to use in different downstream applications.

Procedure for gel extraction:

- excision of the DNA-fragment from the agarose gel with a sharp scalpel
- gel removal and binding of DNA fragments on the membrane of the spin column
- selective binding of DNA fragments to the surface of the DNA-Binding Spin Filter
- elimination of contaminants and ethanol
- elution of the highly pure DNA fragment

Sampling and storage of starting material

Use low melting and standard gels (0.8 – 2%) with TAE or TBE buffer systems. Best results are obtained using freshly prepared DNA slices from a gel. However, the samples can be stored for some weeks at 4 - 8°C.

Excision of DNA fragments

For best results, the pieces of gel should be as small as possible. Do not expose the gel with the DNA fragment to UV light for a long time, reduce cutting time under UV light to a minimum since it is damaging to the DNA**.

Gel removal and binding of DNA fragments

Gel Solubilizer S in the **Invisorb® Fragment CleanUp** solubilizes the agarose gel slice under high temperatures. Together with Binding Enhancer and Binding Buffer, it provides the appropriate condition for the binding of the DNA to the silica membrane at high salt concentrations.

Removal of contaminants and of Ethanol

The DNA fragments bind to the membrane, contaminants and salts are washed away by the Ethanol-containing Wash Buffer. Any remaining Wash Buffer, which may interfere with subsequent enzymatic reactions, is removed by additional centrifugation.

Elution of PCR products or DNA fragments

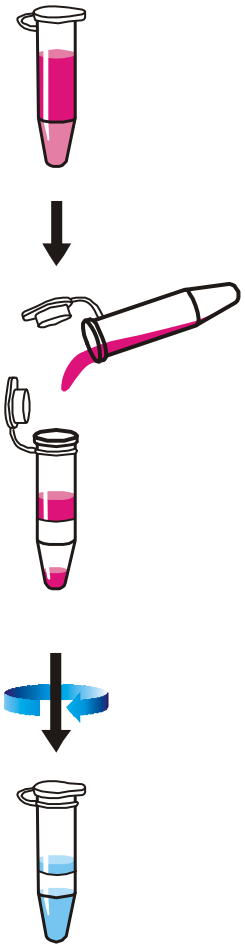
DNA is eluted from the column using 10 - 50 µl Elution Buffer.
Eluting twice each with 30 - 50 µl leads to complete recovery of DNA. By the use of smaller elution volumes DNA concentration can be increased. Elution volumes should not fall below 10 µl, otherwise the yield will be reduced. The eluted DNA is ready to use in different downstream applications.

**) see protocol 5

Preparing reagents and buffers of the Invisorb® Fragment CleanUp

250 preps
Add 105 ml 96-100% Ethanol to each bottle **Wash Buffer** Add 120 ml 99.7% Isopropanol to the **Binding Enhancer;** Mix by intensive shaking for 1 min. Shortly before use mix by inverting several times Add 100 ml 99.7% Isopropanol to the **Binding Buffer;** Mix by intensive shaking for 1 min. Shortly before use mix by inverting several times

Scheme for DNA fragment purification



Please read the protocols carefully prior starting the preparation procedure.

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up to 50 µl PCR-mixtures or enzymatic reaction mixtures
add 250 µl **Binding Buffer** to the PCR sample
> 50 µl up to 100 µl PCR-mixtures or enzymatic reaction mixtures
add 500 µl **Binding Buffer** *(follow preparing instructions)* to the PCR sample

mix very well by pipetting up and down or vortexing

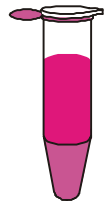
transfer the sample completely onto the provided Spin Filter
incubate for 1 minute at room temperature
centrifuge for 4 min at maximum speed

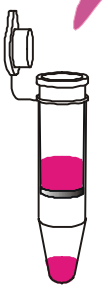
place the Spin Filter into a new 1.5 ml Receiver Tube
add at least 10 µl **Elution Buffer** (or ddH_20) directly onto the center of the Spin Filter

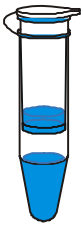
incubate for 1 minute at room temperature
centrifuge for 1 minute at 11.000 x g (11.000 rpm)

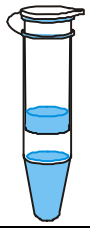
DNA in the eluate is now ready to use

Scheme for agarose gel extraction









Please read the protocols carefully prior starting the preparation procedure.

Transfer gel slices (max. 300 mg) into a 1.5 ml or 2.0 ml microcentrifuge tube (not provided)

to gel slices up to 150 mg add 500 µl **Gel Solubilizer S**
to gel slices > 150 mg add 1 ml of **Gel Solubilizer S**

incubate at 50°C for 10 minutes until the gel is completely solubilized

add 250 µl **Binding Enhancer** *(follow preparing instructions)* to a 500 µl reaction volume
add 500 µ**l Binding Enhancer** *(follow preparing instructions)* to a 1 ml reaction volume

mix by pipetting up and down for 2-3 times

load approx. 800 µl of the sample onto the Spin Filter
centrifuge at 11.000 x g (11.000 rpm) for 2 min
discard the filtrate
for reaction volumes > 800 µl reload the residual volume and repeat the centrifugation step

add 500 µl **Wash Buffer** to the Spin Filter
centrifuge for 1 min at 11.000 x g (11.000 rpm)
discard the filtrate
repeat the washing step once again
centrifuge for 1 min at 11.000 x g (11.000 rpm), discard the filtrate

remove the remaining ethanol by centrifugation for 4 min at maximum speed

transfer the Spin Filter into a new 1.5 ml Receiver Tube
add at least 10 µl **Elution Buffer** directly to the center of the Spin Filter
incubate at room temperature for 1 min
centrifuge for 1 minute at 11.000 x g (11.000 rpm)

DNA is now ready to use

Protocol 1: Purification and concentration of DNA fragments from enzymatic reactions, e.g. PCR-products from PCR reactions, cDNA synthesis, enzyme restriction digestions

Please read the instructions carefully and carry out preparatory arrangements in advance.

Note: *Before starting with the purification procedure please place a Spin Filter into a 2.0 ml Receiver Tube!*

Attention: *Please prepare the* ***Binding Buffer*** *ahead- see instruction page: 11*

1. Binding of the PCR or DNA - fragments

A. For PCR-mixtures up to 50 µl

Add **250 µl Binding Buffer** to the PCR sample and mix very well by pipetting or vortexing. Transfer the sample completely to a Spin Filter, incubate for 1 minute at room temperature and centrifuge for 4 min at maximum speed.

B. For PCR-mixture > 50 µl up to 100 µl

Add **500 µl Binding Buffer** to the PCR sample and mix very well by pipetting or vortexing. Transfer the sample completely onto a Spin Filter, incubate for 1 minute at room temperature and centrifuge for 4 min at maximum speed.

2. Elution of the PCR or DNA - fragments

Place the Spin Filter into a new 1.5 ml Receiver Tube.
Add at least 10 µl Elution Buffer (or ddH_20) directly onto the center of the Spin Filter.
Incubate for 1 minute at room temperature. Centrifuge for 1 minute at 11.000 x g (11.000 rpm).

Important notes:

1. If the PCR-mixture contains mineral oil, we recommend the addition of 500 µl of Binding Buffer independent of the starting volume. It is also possible to wash the bound PCR-fragment once with 500 µl of Binding Buffer.

2. To increase the final DNA yield we recommend an extended incubation time with Elution Buffer (up to 5 minutes), which will lead to a slightly higher final yield.

3. For concentration of PCR-fragments it is possible to elute with lower volume of Elution Buffer, than the volume of the starting PCR-mixture. The minimum volume is 10 µl.

4. For ligation mixtures please note, that ligation reactions give very often unwanted side products. These are purified and enriched as well.

Protocol 2: Removal of DyeDeoxy™ terminators from DNA cycle sequencing reactions of PCR-products and plasmids after use ABI Prism™ terminator Kits

Please read the instructions carefully and carry out preparatory arrangements in advance.

Note: *Before starting with the purification procedure please place a Spin Filter into a 2.0 ml Receiver Tube!*

Attention: *Please prepare the* ***Binding Buffer*** *ahead-* *see instruction page: 11*

1. Binding of the (fluorescent) labeled DNA

Add **500 µl Binding Buffer** to the completed cycle sequencing reaction (up to 100 µl) and mix thoroughly by pipetting or vortexing. Transfer the sample completely onto a Spin Filter and centrifuge for 4 min at maximum speed.

Note:
If sequences next to the primer (short fragments) shall be obtained, the addition of up to 150 µl of Isopropanol to the upper mixture may be helpful (the shorter the desired fragments are the more Isopropanol must be used). This leads to lower purity but also to recovery of shorter fragments.

2. Elution of the (fluorescent) labeled DNA

Place the Spin Filter into a new 1.5 ml Receiver Tube.
Add at least 10 µl Elution Buffer (or ddH_20) directly onto the center of the Spin Filter.
Incubate for 1 minute at room temperature. Centrifuge for 1 minute at 11.000 x g (11.000 rpm).
Discard the Spin Filter and proceed with the ABI sample loading.

Protocol 3: Extraction of DNA fragments from agarose gel slices

Please read the instructions carefully and carry out preparatory arrangements in advance.

Important: *TBE-gels contain more potentially inhibitors for downstream application than TAE-gels. Therefore, we recommend the use of TAE-gels for critical downstream applications! Before starting with the purification procedure please place a Spin Filter into a 2.0 ml Receiver Tube!*

Attention: *Please prepare the* ***Binding Buffer*** *ahead - see instruction page: 11*

1. Excise the DNA-fragment from 0.8 – 2% agarose gel with a sharp scalpel. The gel piece should be cut out as small as possible. Prevent long exposure to UV light. Verify the weight of the piece.

For gel slices up to 150 mg add 500 µl Gel Solubilizer S.
For gel slices > 150 mg – 300 mg add 1 ml of Gel Solubilizer S.

Do not use more than 300 mg gel slice for one Spin Filter.
Transfer the gel slice into a 1.5 or 2.0 reaction tube.

2. Incubate at 50°C for 10 minutes until the agarose gel slice is completely dissolved. Continuous shaking during incubation (e.g. Eppendorf Thermo mixer) is very helpful.

3. Add 250 µl Binding Enhancer to a 500 µl reaction volume or 500 µl Binding Enhancer to a 1 ml reaction volume and mix the suspension by pipetting or by vortexing.
Load approx. 800 µl of the sample onto the Spin Filter. Centrifuge at 11.000 x g (11.000 rpm) for 2 minutes. Discard the filtrate. For reaction volumes > 800 µl reload the remaining volume onto the Spin Filter and repeat the centrifugation step.

4. Add 500 µl Wash Buffer to the Spin Filter and centrifuge for 1 min at 11.000 x g (11.000 rpm). Discard the filtrate. Repeat the washing step once again.

5. Discard the filtrate. Remove the residual ethanol of the Wash Buffer by centrifugation for 4 min at maximum speed.

6. Transfer the Spin Filter to a new 1.5 ml Receiver Tube.
Add at least 20 µl Elution Buffer directly to the center of the Spin Filter.
Incubate at room temperature for 1 minute. Centrifuge for 1 minute at 11.000 x g (11.000 rpm).

Note: *To increase the final DNA yield we recommend using a higher volume of Elution Buffer. Please take into account that an increasing volume of Elution Buffer reduces the final concentration of the purified DNA.*
An extended incubation time with Elution Buffer (up to 10 minutes) leads also to a slightly higher final yield.

Additional Protocol 4: Purification of PCR - products from 200 µl PCR reactions

Please read the instructions carefully and carry out preparatory arrangements in advance.

Note: *Before starting with the purification procedure please place a Spin Filter into a 2.0 ml Receiver Tube!*

Attention: *Please prepare the* ***Binding Buffer*** *ahead- see instruction page: 11*

1. Binding of the PCR-fragments

For PCR-reaction of 200 µl

Add **1000 µl Binding Buffer** to the PCR sample and mix thoroughly by pipetting or vortexing. Transfer the sample in two aliquots onto a Spin Filter and centrifuge for 1 min at 11.000 x g (11.000 rpm) each. Remove the filtrate and centrifuge again for 4 minutes at maximum speed.

2. Elution of the PCR-fragments

Place the Spin Filter into a new 1.5 ml Receiver Tube.
Add at least 10 µl Elution Buffer (or ddH_20) directly onto the center of the Spin Filter.
Incubate for 1 minute at room temperature. Centrifuge for 1 minute at 11.000 x g (11.000 rpm).

Important Notes:

1. *The provided volume of Binding Buffer is calculated based on the required buffer volumes in protocol 1 and 2. The amount needed for protocol 4 is not considered.*
2. *If the PCR-mixture contains mineral oil, we recommend the addition of 500 µl Binding Buffer independent of the starting volume. It is also possible to wash the bound PCR fragment once with 500 µl of Binding Buffer.*
3. *To increase the final DNA yield we recommend an extended incubation time with Elution Buffer (up to 5 minutes), which will lead to a slightly higher final yield.*
4. *For concentration of PCR-fragments, it is possible to elute with lower volume of Elution Buffer than the volume of the starting PCR-mixture. The minimum volume is 10 µl.*

Additional Protocol 5: Instruction for adding A-overhangs to PCR products after gel purification

Please read the instructions carefully and carry out preparatory arrangements in advance.

Transfer 30 µl of the extracted PCR product to a 1.5 ml reaction tube.

Add 2 units standard Taq DNA Polymerase (no proofreading activity), 3.5 µl Taq Buffer, 0.6 µl dNTPs (10 mM each), if not contained in the Taq Buffer in a 35 µl reaction volume

Incubate for 15 min at 72°C under continuous shaking in a thermomixer.

After this treatment fragments can be used in cloning/ligation experiments.

Troubleshooting for agarose gel extraction

Problem	Cause	Comments and suggestions
Low recovery	No ethanol added to the **Wash Buffer**	Prepare the **Wash Buffer** exactly as described in the manual. Store the **Wash Buffer** with firmly fixed lid.
	Poor elution of DNA, ineffective solubilization of the agarose gel slice	Add the **Elution Buffer** directly onto the center of the Spin Filter (also if a small elution volume is used). For smaller fragments than 500 bp, please use TAE agarose gels.
	No **Binding Enhancer** added	Avoid this mistake.
Problems with down stream application, e.g. ligation	Contamination with salt components Contamination with agarose traces	The gel slice must be completely dissolved. Add the amount of **Binding Enhancer** needed to the solubilized suspension. Wash off the Spin Filters as described in the manual. Prolong the incubation time with **Wash Buffer** to 5 minutes before centrifugation.
	Contamination of the final DNA with ethanol	Keep the given centrifugation time, extend it if necessary (verify the smell).

Troubleshooting for DNA fragment purification

Problem	Cause	Comments and suggestions
Low recovery	Poor elution of DNA	Add the **Elution Buffer** directly onto the center of the Spin Filter (even if a small elution volume is used).
	Problems with mineral oil	Try to avoid pipetting of mineral oil. Apply the correct centrifugation steps. Take a higher volume of **Binding Buffer.** Wash once with **Binding Buffer.**

Ordering information

Product	Package Size	Catalogue No.
Invisorb® Fragment CleanUp	250 purifications	1020300300

Possible suppliers for Isopropanol

Carl Roth
2-Propanol
Rotipuran ≥99.8%, p.a., ACS, ISO
Ordering No. 6752

Applichem
2-Propanol für die Molekularbiologie
Ordering No. A3928

Sigma
2-Propanol
Ordering No. 59304-1L-F

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