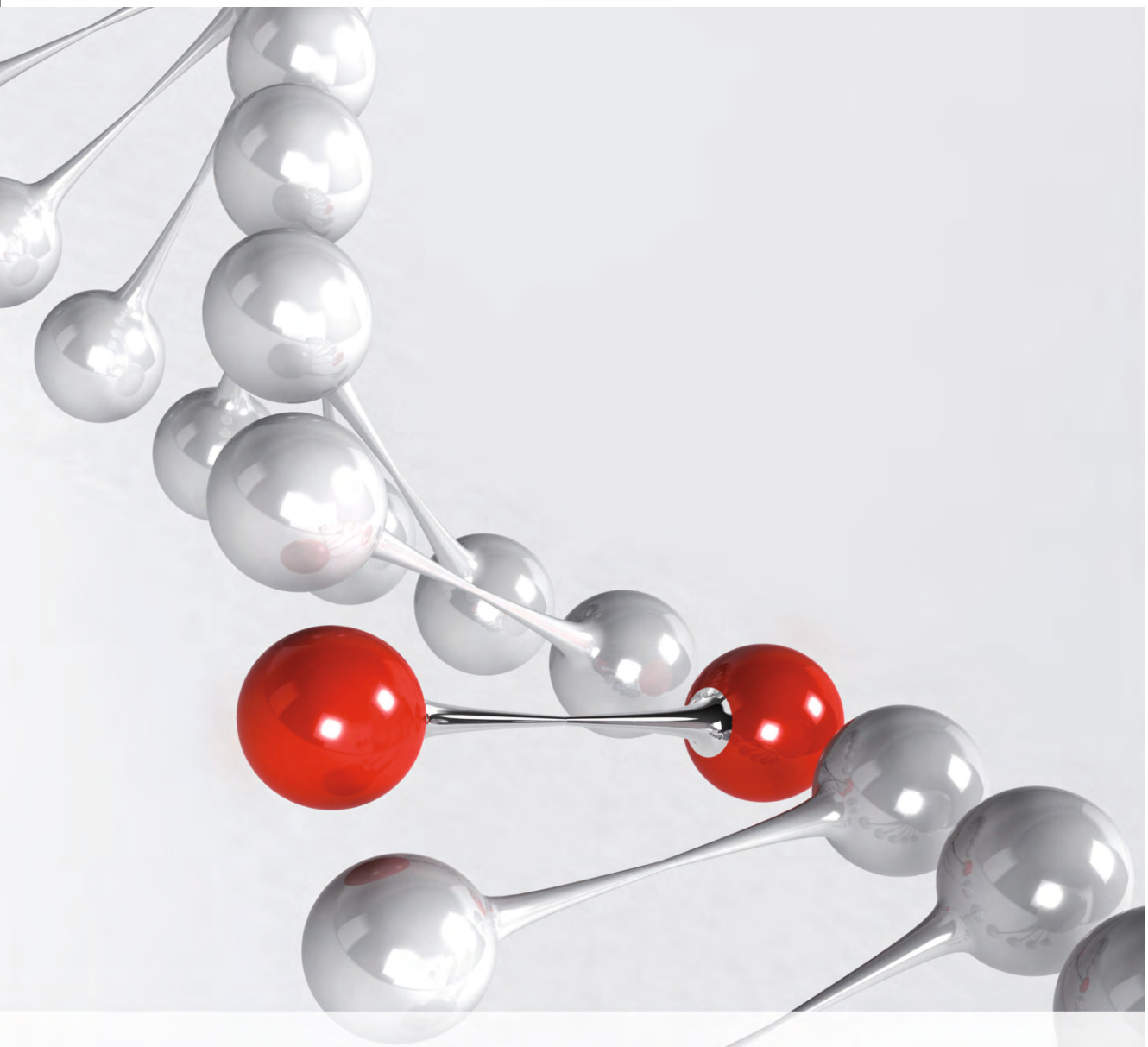
stratec molecular



User manual

MSB® Spin PCRapace *
Invisorb® Spin DNA Extraction Kit **
Invisorb® Fragment CleanUp***

REF 1020220x00*
1020110x00**
1020300x00***

STRATEC Molecular GmbH, D-13125 Berlin

for ultra fast purification of DNA fragments after PCR and other enzymatic reactions or/ and for the extraction of DNA fragments from agarose gels

Instruction for the

MSB® Spin PCRapace

With only 5 min of processing time, the **MSB® Spin PCRapace** is the fastest system for purification of up to 100 µl PCR products (80 bp - 30 kb) from dNTP's, primers, enzymes, additives and salts using the unique **MSB® technology** – a washing step is not required.
The kit is useful as well for the cleanup of DNA fragments from salts and enzymes from restrictions digestion, ligation and cDNA synthesis mixtures. For the efficient concentration of PCR products the **MSB® Spin PCRapace** can be used as well as for the reliable removal of Dye terminators from DNA cycle sequencing reactions. The recovery of PCR product is 80 – 95 %.

Invisorb® Spin DNA Extraction

The **Invisorb® Spin DNA Extraction Kit** is the ideal tool for extraction of DNA fragments of 80 bp – 30 kb from standard or low melt agarose gels in TAE and TBE buffers in high end-concentration of DNA. Up to 300 mg agarose gel slices can be processed per spin column.

Invisorb® Fragment CleanUp

The **Invisorb® Fragment CleanUp** is a combination of different technologies.

Using **MSB® technology** the kit is the ideal tool for ultra fast purification of DNA fragments (80 bp – 30 kb) after PCR and other enzymatic reactions, for the efficient concentration of DNA fragments as well as for the reliable removal of Dye terminators from DNA cycle sequencing reactions. The recovery of PCR product is 80 – 95 % and a washing step is not required
Using the **Invisorb® technology** the kit is the ideal tool for the efficient extraction of DNA fragments from TAE or TBE agarose gels.

Contents

Kit contents of the MSB® Spin PCRapace

Store all kit components at room temperature.

	10 preps	50 preps	250 preps	500 preps
Catalogue No.	1020220900	1020220200	1020220300	1020220400
Binding Buffer	4 ml (final volume 11 ml)	12 ml (final volume 32 ml)	63 ml (final volume 163 ml)	2 x 63 ml (final volume 2 x 163 ml)
Elution Buffer	2 ml	3 x 2 ml	30 ml	60 ml
Spin Filter	10	50	5 x 50	10 x 50
2.0 ml Receiver Tubes	10	50	5 x 50	10 x 50
1.5 ml Receiver Tubes	10	50	5 x 50	10 x 50
Manual	1	1	1	1
Initial steps	add 7 ml 99.7% Isopropanol to the Binding Buffer	add 20 ml 99.7% Isopropanol to the Binding Buffer	add 100 ml 99.7% Isopropanol to the Binding Buffer	add 100 ml 99.7% Isopropanol to each Binding Buffer

Kit contents of the Invisorb® Spin DNA Extraction Kit

Store all kit components at room temperature.

	5 preps	50 preps	250 preps
Catalogue No.	1020110100	1020110200	1020110300
Gel Solubilizer S	3 x 2 ml	60 ml	2 x 140 ml
Binding Enhancer	2 ml (final volume 10 ml)	6 ml (final volume 30 ml)	3 x 10 ml (final volume 50 ml)
Wash Buffer	15 ml (ready to use)	18 ml (final volume 60 ml)	2 x 45 ml (final volume 2 x 150 ml)
Elution Buffer	2 ml	2 x 2 ml	15 ml
Spin Filter	5	50	5 x 50
2.0 ml Receiver Tubes	5	50	5 x 50
1.5 ml Receiver Tubes	5	50	5 x 50
Manual	1	1	1
Initial steps	add 8 ml 99.7% Isopropanol to the **Binding Enhancer**	add 24 ml 99.7% Isopropanol to the **Binding Enhancer** add 42 ml 96-100% ethanol to the bottle **Wash Buffer**	add 40 ml 99.7% Isopropanol to each **Binding Enhancer** add 105 ml 96-100% ethanol to each bottle **Wash Buffer**

Kit contents of Invisorb® Fragment CleanUp

Store all kit components at room temperature.

	10 preps	50 preps	250 preps
Catalogue No.	1020300900	1020300200	1020300300
Gel Solubilizer S	12 ml	60 ml	2 x 140 ml
Binding Buffer	4 ml (final volume 11 ml)	12 ml (final volume 32 ml)	63 ml (final volume 163 ml)
Binding Enhancer	2 ml (final volume 10 ml)	6 ml (final volume 30 ml)	30 ml (final volume 150 ml)
Wash Buffer	15 ml (ready to use)	18 ml (final volume 60 ml)	2 x 45 ml (final volume 2 x 150)
Elution Buffer	2 ml	2 x 2 ml	15 ml
Spin Filter	10	50	5 x 50
2.0 ml Receiver Tubes	10	50	5 x 50
1.5 ml Receiver Tubes	10	50	5 x 50
Manual	1	1	1
Initial steps	add 8 ml 99.7% Isopropanol to the **Binding Enhancer** add 7 ml 99.7% Isopropanol to the **Binding Buffer**	add 42 ml 96-100% ethanol to the bottle **Wash Buffer** add 24 ml 99.7% Isopropanol to the **Binding Enhancer** add 20 ml 99.7% Isopropanol to the **Binding Buffer**	add 105 ml 96-100% ethanol to each bottle **Wash Buffer** add 120 ml 99.7% Isopropanol to the **Binding Enhancer** add 100 ml 99.7% Isopropanol to the **Binding Buffer**

Symbols

 Manufacturer

 Lot number

 Catalogue number

 Expiry date

 Consult operating instructions

 Temperature limitation

 Do not reuse

Storage

All buffers of the **MSB® Spin PCRapace, Invisorb® Spin DNA Extraction Kit** and the **Invisorb® Fragment CleanUp** should be stored well sealed and dry at room temperature and are stable for at least 12 months under these conditions.

Before every use make sure that all components are at room temperature. If there are any precipitates within the provided solutions dissolve these precipitates by warming up carefully.

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

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **MSB® Spin PCRapace, Invisorb® Spin DNA Extraction Kit** and the **Invisorb® Fragment CleanUp** 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, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the Product free of charge.

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

In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **MSB® Spin PCRapace, Invisorb® Spin DNA Extraction Kit** and the **Invisorb® Fragment CleanUp** 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 **MSB® Spin PCRapace, Invisorb® Spin DNA Extraction Kit** and the **Invisorb® Fragment CleanUp** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

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.

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.stratec.com** for each STRATEC 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.

STRATEC Molecular has not tested the liquid waste generated by the **MSB® Spin PCRapace, Invisorb® Spin DNA Extraction Kit** and the **Invisorb® Fragment CleanUp** procedures 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 discarded according to local safety regulations.

European Community risk and safety phrases for the components of the **MSB® Spin PCRapace, Invisorb® Spin DNA Extraction Kit** and the **Invisorb® Fragment CleanUp** to which they apply are listed below as follows:

Gel Solubilizer

warning
H302-312-332-412 EUH032 P273

H302:	Harmful if swallowed.
H312:	Harmful in contact with skin.
H332:	Harmful if inhaled.
H412:	Harmful to aquatic life with long lasting effects.
EUH032:	Contact with acids liberates very toxic gas.
	present and easy to do. Continue rinsing.
P273:	Avoid release to the environment.

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**

Intended use

See for each product the product characteristic, there are presented the intended use and the product use limitation for each kit!

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 RNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.
To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

The kits are developed, designed, and sold for research purposes only.

Product use limitation

For purification the fragments should not contain less than 70 bp.
The maximum length of primers witch can removed is 40 bp.
DNA fragments should not be bigger than 30 kb and not smaller than 70 bp.
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 STRATEC Molecular Product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the Product with respect to specific applications. STRATEC Molecular Products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All Products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC 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.

PCR product & DNA fragment purification and concentration

This manual characterizes innovative kits for membrane adsorption based purification of PCR products or DNA fragments from enzymatic reaction mixtures and of DNA fragments from agarose gels using the high-performance **MSB®** or the well established **Invisorb® technology**. Different kits were developed and adapted to suit different DNA fragment purification needs.

Sample Source	**Recommended Kit*****	**Sample Volume**	**Recovery**
PCR products, ligation reaction mixture, enzyme digestion mixture, cDNA synthesis mixture cycle sequencing reaction mixture	MSB® Spin PCRapace Invisorb® Fragment CleanUp	up to 100 µl	80 – 95 %*
Sample Source	**Recommended Kit*****	**Sample Volume**	**Recovery**
Strong contaminated DNA, DNA fragments and PCR products	Invisorb® DNA CleanUp (see ordering information page 28)	up to 200 µl	60 - 85 %*
Agarose gels (TAE, TBE)	Invisorb® Spin DNA Extraction Invisorb® Fragment CleanUp	up to 300 mg gel slices	60 – 90 %**

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 purification procedure has been optimized to remove salts, enzymes, nucleotides, mineral oil, 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 characterized on the following pages.

MSB® technology

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

This development opens new possibilities for simplifying the purification procedure and to considerably reduce handling steps and processing time. The **MSB® kits** have been designed for extremely efficient purification and/or concentration of PCR products or of DNA fragments from enzymatic reaction mixtures with **only two steps**.

The DNA fragments bind to the membrane at minimal concentrations of non chaotropic salts. Therefore a washing step is not required. High concentrated, pure DNA fragments are eluted ready to use.

Advantages:

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

Invisorb® technology

is the first technology for the extraction of highly purified nucleic acids using non chaotropic binding conditions.

Starting from complex biological samples, the method allows binding of nucleic acids to nearly all surfaces, such as membranes, carrier or magnetic particles. In combination with specially modified surfaces a selective binding of different nucleic acid targets can be realized. Binding of nucleic acids under non chaotropic salt conditions leads to

- high binding efficiency
- reproducible high yields of ready to use DNA simplified protocols; reliable, time saving and easy handling steps
- guaranteed freedom of operation for all fields of applications
- improved quality assurance in the customers laboratories

Product characteristics of the MSB® Spin PCRapace

Starting material	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. 5 min

The **MSB® Spin PCRapace** provides a convenient tool for ultra fast and efficient direct purification of about 100 µl PCR products from 80 bp up to 30 kb from amplification reactions. With max. 5 min of processing time the **MSB® Spin PCRapace** is the fastest system for the separation of PCR products (80 bp - 30 kb) from dNTP's, primers, enzymes, additives, labelling reagents (biotin, radioactive ATP etc.) and salts. The recovery of PCR product is 80 – 95 %. The kit is further useful for DNA fragment cleanup from

- restriction digestion mixture
- ligation mixture
- cDNA synthesis mixtures

The kit is also a powerful and efficient tool for the

- concentration of DNA fragments as well as
- for purification of linearized pDNA from restriction mixtures and
- for the reliable removal of Dye terminators from DNA cycle sequencing reactions

The DNA-fragments will be bound directly onto the surface of a spin filter column based on new buffer composition. No additional and common used washing steps are necessary. Finally, the DNA fragments will be eluted with low salt buffer or H_2O.

MSB® Spin PCRapace	parameters
Binding capacity for fragment size of 200 – 500 bp Binding capacity for fragment size of 20.000 kb Elution volume	up to 10 µg up to 100 µg minimum 10 µl

Beside the extremely time efficient procedure the kits provides high yield and purity of the recovered DNA fragments. The recovery rate (80 - 95 %) is nearly independent from PCR additives, the PCR reaction volume and the fragment size. The purified PCR product can be used in subsequent downstream applications:

- sequencing
- cloning and labeling experiments
- hybridization
- transcription
- digestion with restriction enzymes

Product use limitation

The kit works not suitable with PCR products smaller than 70 bp, single stranded DNA may diverge.

Product characteristics of the Invisorb® Spin DNA Extraction Kit

Starting material **Size of DNA fragments: 80 bp - 30 kb**	**Yield**	Time for preparation
up to 300 mg of gel slices of from TAE or TBE buffered systems	70 - 90 %, depends on fragment length and kind of agarose gel	approx. 20 min

The **Invisorb® Spin DNA Extraction Kit** provides a convenient tool for fast and efficient extraction and purification of DNA fragments directly from TAE and TBE agarose gels slices in less than 20 min in the comfortable spin filter format. The purification procedure removes agarose, ethidium bromide and other impurities from the DNA sample.

After gel solubilization DNA fragments from 80 bp up to 30 kb are bound directly onto the membrane of a spin filter column. All impurities are removed very efficiently by a washing step. The DNA is eluted in as little as 20 µl low-salt buffer.

Invisorb® Spin DNA Extraction Kit	**parameters**
Binding capacity for fragment size of 200 – 500 bp Binding capacity for fragment size of 20.000 kb Elution volume	up to 10 µg up to 100 µg minimum 20 µl

The handling and the composition of the **Invisorb® Spin DNA Extraction Kit** is optimized to guarantee high recovery and purity of the DNA fragments. The purified DNA fragments are ready to use for various downstream applications

- digestion with restriction enzymes
- hybridization, labeling, cloning
- sequencing
- *in vitro* Transcription

Product use limitation

It is not suitable for purification of circular plasmids, because as a result of their very different configurations they were detected in the gel in many positions. Furthermore purification of linearized pDNA is possible.

Important Notes

If processing PCR with Taq-Polymerase, possibly the “a overhangs” can become lost during the extraction. It is recommended to perform reparation of a overhangs subsequent to the extraction if they are needed in the following downstream application. (see page 26)

Product characteristics of the Invisorb® Fragment CleanUp

The **Invisorb® Fragment CleanUp** is a combination of **MSB® Spin PCRapace** and **Invisorb® Spin DNA Extraction Kit.**

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. 5 min
up to 300 mg of gel slices of from TAE or TBE agarose gels	70 - 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 from 80 bp up to 30 kb and of other enzymatic reaction mixtures, or of linearized pDNA as well as for the purification of DNA-fragments from agarose gels.

For PCR cleanup the MSB® procedure is offered -no commonly used washing steps are necessary. Finally, the DNA fragments will be eluted with low salt buffer or H_2O.

For purification of DNA-fragments from agarose gels the Invisorb® procedure is offered. The DNA fragments are bound directly onto the surface of a spin filter column after gel solubilization. The DNA – fragments will be eluted in a low salt buffer after washing.

The extraction protocol as well as all buffers is optimized to provide high yield and purity of the recovered DNA-fragment. The “hands-on time“necessary for the whole procedure is reduced to a minimum. The purification process will be ready in 5 - 20 minutes. 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, microinjection

Product use limitation

It is not suitable for extraction of circular plasmids, because as a result of their very different configurations they were detected in the gel in many positions.
If processing PCR with Taq-Polymerase, possibly the “a overhangs” can become lost during the extraction. It is recommended to perform reparation of a overhangs subsequent to the extraction if they are needed in the following downstream application. (see page 26)

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 STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 6). 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, 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 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 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). (See our webpage: www.stratec.com)

- Microcentrifuge (≥ 11.100 x g (11.000 rpm)
- Ethanol (96-100%)
- Thermoshaker
- Pipettes and filter tips
- Scalpel 120
- 1.5 ml and 2.0 ml reaction rubes
- Isopropanol

*The **MSB® Spin PCRapace, Invisorb® Spin DNA Extraction Kit** and the **Invisorb® Fragment CleanUp** are 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
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 MSB® Spin PCRapace

The **MSB® Spin PCRapace** procedure comprises the following steps:

- selective binding of DNA fragments to the surface of the DNA-Binding Spin Filter
- elimination of contaminants and ethanol
- elution of highly pure DNA fragment or PCR product

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 range of 70 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, are drawn through the column by centrifugal force together with the big 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

The efficiency of elution strongly depends on the concentration of salt and on the pH of the elution buffer. Unlike absorption, elution is most efficient under basic conditions and low concentrations of salt. High concentrated, pure DNA fragments are eluted with a minimum of 10 µl of Tris buffer or water. Maximum elution efficiency is achieved between pH 7.0 and 8.5; therefore, when using water to elute, be certain that the pH is within this range. DNA must also be stored at –20 °C when eluted with water, as it is possible that DNA may degrade without a buffering agent.

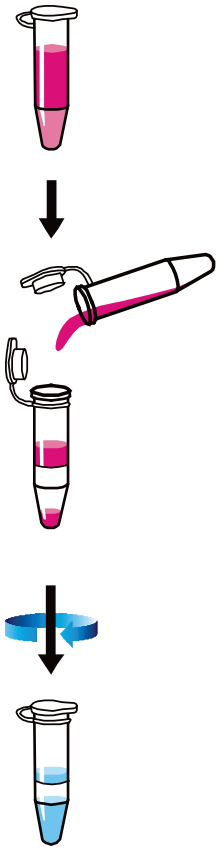
Sampling and storage of starting material

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°C .

Preparing reagents and buffers of the MSB® Spin PCRapace

10 preps:
add 7 ml 99.7% Isopropanol to the Binding Buffer
50 preps:
add 20 ml 99.7% Isopropanol to the Binding Buffer
250 preps
add 100 ml 99.7% Isopropanol to the Binding Buffer
500 preps:
add 100 ml 99.7% Isopropanol to each Binding Buffer

Scheme of MSB® Spin PCRapace



Please read the protocols carefully prior to the start of the preparation procedure

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
centrifuge for 2 min at 11.000 x g (11.000 rpm).
remove the filtrate and centrifuge again for 3 minutes

place the Spin Filter into a new 1.5 ml Receiver Tube
add at least 10 µl Elution Buffer (or ddH20)
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

Principle and procedure of the Invisorb® Spin DNA Extraction Kit

The **Invisorb® Spin DNA Extraction Kit** procedure comprises the following steps:

- excise of DNA-fragments 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 highly pure DNA fragment

Sampling and storage of starting material

Best results are obtained using freshly prepared DNA slices from gel. 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. It is damaging to the DNA. The samples can be stored for some weeks at 4°C .

Excise of DNA fragments

For best results the pieces of gel should be small as possible.
Use low melting and standard gels with TAE or TBE buffer systems.

Gel removal and binding of DNA fragments

Gel Solubilizer S in the **Invisorb® Spin DNA Extraction Kit** solubilizes the agarose gel slice under higher temperatures. Together with Binding Enhancer and Binding buffer, it provides the appropriate condition for the binding of DNA to the silica membrane under high concentrations of salt.

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 DNA fragments

The efficiency of elution strongly depends on the concentration of salt and on the pH of the elution buffer. Unlike to absorption, elution is most efficient under basic conditions and low concentrations of salt. High concentrated, pure DNA fragments are eluted with a minimum of 10 µl of Tris buffer or water. Maximum elution efficiency is achieved between pH 7.0 and 8.5; therefore, when using water to elute, be certain that the pH is within this range. DNA must also be stored at –20 °C when eluted with water, as it is possible that DNA may degrade without a buffering agent.

DNA yield and concentration

DNA yield is dependent on the following three factors: the volume of elution buffer, how the buffer is applied to the column, and the incubation time of the buffer on the column. Between 100 and 200 µl of the elution buffer completely covers the **Invisorb®** membrane, ensuring maximum yield, even when not applied directly to the center of the membrane. Elution with up to 50 µl requires the buffer to be added directly to the center of the membrane, and an additional 1-2 minute incubation is required for maximum yield if elution is performed with the minimum recommended volume of 10 µl.

DNA fragment purification from agarose gel

- Gel removal and binding of DNA fragments on the membrane of the spin column
- Removal of contaminants and elimination of ethanol
- Elution of DNA fragments

Gel removal and binding of DNA fragments

Gel Solubilizer S in the **Invisorb® Fragment CleanUp Kit** solubilizes the agarose gel slice and, together with the binding enhancer and binding buffer, provides the appropriate condition for the binding of DNA to the silica membrane under high concentrations of salt.

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, agarose, dyes, ethidium bromide, oils, and detergents do not bind to the silica membrane; instead, they flow through the column. Salts are washed away by the ethanol-containing Wash-Buffer PE. Any remaining Wash Buffer, which may interfere with subsequent enzymatic reactions, is removed by additional centrifugation.

Elution of DNA fragments

The efficiency of elution strongly depends on the concentration of salt and on the pH of the elution buffer. Unlike absorption, elution is most efficient under basic conditions and low concentrations of salt. High concentrated, pure DNA fragments are eluted with a minimum of 10 µl of Tris buffer or water. Maximum elution efficiency is achieved between pH 7.0 and 8.5; therefore, when using water to elute, be certain that the pH is within this range. DNA must also be stored at –20 °C when eluted with water, as it is possible that DNA may degrade without a buffering agent.

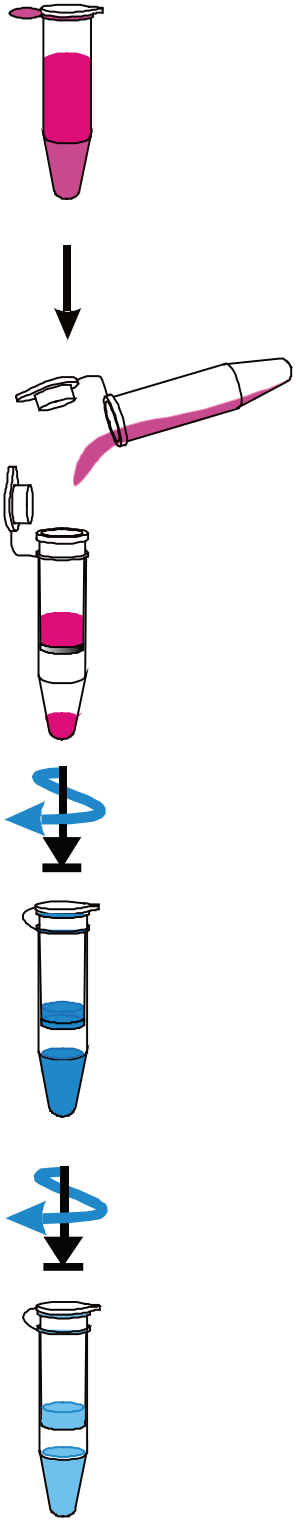
DNA yield and concentration

DNA yield depends on the following three factors: the volume of elution buffer, how the buffer is applied to the column, and the incubation time of the buffer on the column. Between 100 and 200 µl of the elution buffer completely covers the **Invisorb®** membrane, ensuring maximum yield, even when not applied directly to the center of the membrane. Elution with up to 50 µl requires the buffer to be added directly to the center of the membrane, and an additional 1-2 minute incubation is required for maximum yield if elution is performed with the minimum recommended volume of 10µl.

Preparing reagents and buffers of the Invisorb® Spin DNA Extraction Kit

10 preps
add 8 ml 99.7% Isopropanol to the **Binding Enhancer**
50 preps
add 42 ml 96-100% ethanol to the bottle **Wash Buffer** add 24 ml 99.7% Isopropanol to the **Binding Enhancer**
250 preps
add 105 ml 96-100% ethanol to each bottle **Wash Buffer** add 40 ml 99.7% Isopropanol to each **Binding Enhancer**

General Scheme of Invisorb® Spin DNA Extraction Kit



Please read the protocols carefully prior to the start of the preparation procedure

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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**
and 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
and mix by pipetting up and down for 2-3 times

load app. 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 residual ethanol by centrifugation for 4 min at maximum speed

transfer the Spin Filter into a new 1.5 ml Receiver Tube.
add at least 20 µl **Elution Buffer** directly onto the center of the Spin Filter
incubate at room temperature for 5 min
centrifuge for 1 minute at 11.000 x g (11.000 rpm)
DNA is now ready to use

Principle and procedure of the Invisorb® Fragment CleanUp

The Invisorb® Fragment CleanUp Kit combines the convenience of spin-column technology with the selective bonding properties of a uniquely designed silica membrane**.** The Invisorb® columns are designed to give high end-concentrations of purified DNA fragments for subsequent reactions. Special buffers provided with each kit are optimized for efficient recovery of DNA and removal of contaminants in each specific application.

- DNA fragment purification from agarose gel
- Cleanup of PCR mixture or of enzymatic reaction mixtures
- Two handling options: spin columns can be processed in a micro centrifuge or on an Invisorb® 96 Vacuum Manifold

During DNA cleanup using the **Invisorb® Fragment CleanUp Kit,** all enzymes are removed, independent of size and secondary structure.

Protein	**Molecular weight per enzyme subunit (kDa)**
DNA Polymerase I	109
Klenow fragment	62
Calf intestinal alkaline phosphatase (CIP)	69
T4 DNA ligase	55
T4 Polynucleotide kinase	35
Terminal transferase	32
DNase I	31

No additional and commonly used washing and drying steps are necessary. Finally, the DNA fragments will be eluted with low salt buffer or H_2O.

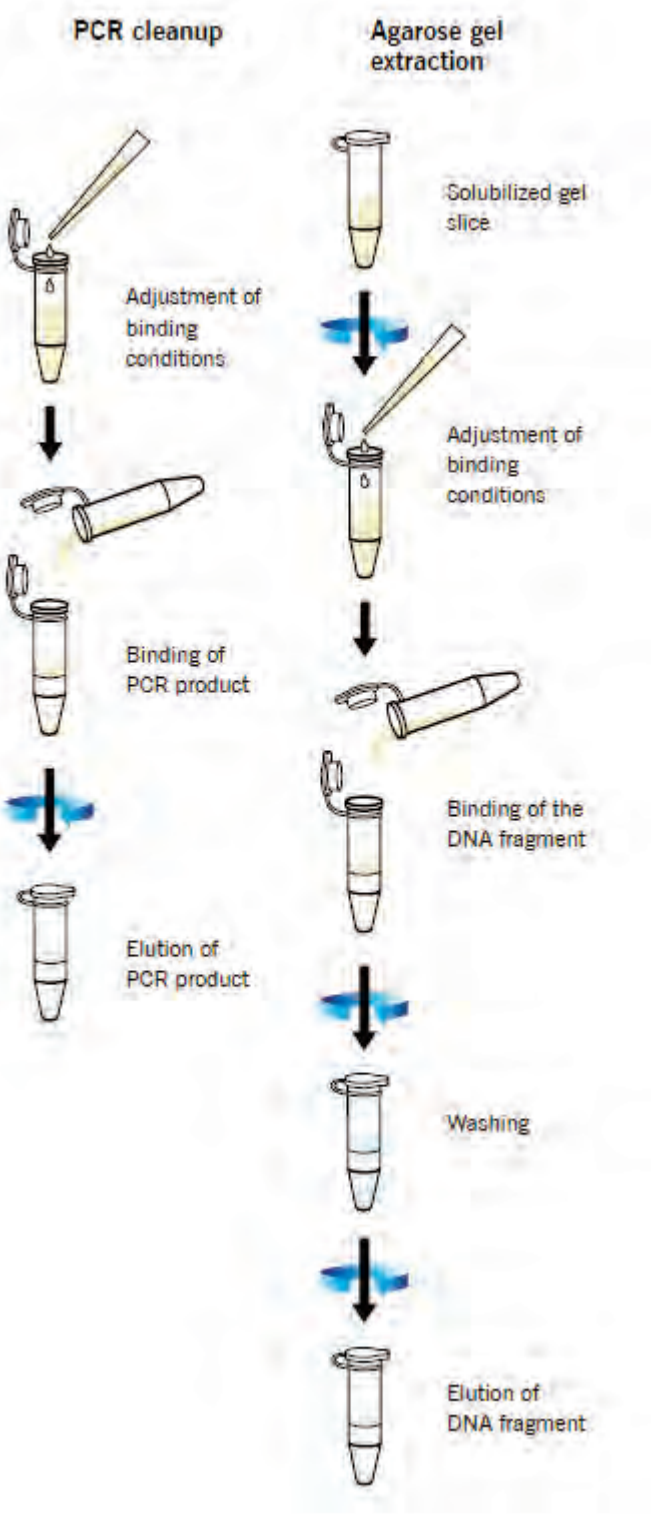
For purification of DNA-fragments from agarose gels, the DNA fragments from 80 bp up to 30 kb will be bound directly onto the surface of a spin filter column after gel solubilization. The DNA – fragments will be eluted in a low salt buffer after washing.
The extraction protocol as well as all buffers is optimized to provide high yield and purity of the recovered DNA-fragment. The “hands-on time“ necessary for the whole procedure is reduced to a minimum. The purification process will be ready in 5 - 20 minutes.
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
- Radioactive and fluorescent sequencing
- Amplification
- Microinjection

Preparing reagents and buffers of the Invisorb® Fragment CleanUp

10 preps
add 8 ml 99.7% Isopropanol to the **Binding Enhancer** add 7 ml 99.7% Isopropanol to the **Binding Buffer**
50 preps
add 42 ml 96-100% ethanol to the bottle **Wash Buffer** add 24 ml 99.7% Isopropanol to the **Binding Enhancer** add 20 ml 99.7% Isopropanol to the **Binding Buffer**
250 preps
add 105 ml 96-100% ethanol to each bottle **Wash Buffer** add 120 ml 99.7% Isopropanol to the **Binding Enhancer** add 100 ml 99.7% Isopropanol to the **Binding Buffer**

General Scheme of Invisorb® Fragment CleanUp



for detailed information see page 16 and 19

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 conduct the prepared procedure!

for MSB® Spin PCRapace and Invisorb® Fragment CleanUp

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

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer*** *– see instruction page: 14*

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 onto a Spin Filter and centrifuge for 2 min at 11.000 x g (11.000 rpm).

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 and centrifuge for 2 min at 11.000 x g (11.000 rpm). Remove the filtrate and centrifuge again for 3 minutes.

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. Centrifugation 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 non wanted side products. These also are purified and enriched.

*** You can order additional Binding Buffer (Cat. No. 10202220) for this application by phone (++49 (0)30 948928910/ 01).**

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 conduct the prepared procedure!

for MSB® Spin PCRapace and Invisorb® Fragment CleanUp

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

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

1. Binding of the (fluorescent) labeled DNA

Add **500 µl Binding Buffer** to the completed cycle sequencing reaction (20 – 100 µl) and mix very well by pipetting or vortexing. Transfer the sample completely onto a Spin Filter and centrifuge for 4 min at maximum speed.

Attention: ***Optional Step 1 if you need primer close by sequence, than perform step one like described below***

This step may help if sequence has to be readable close up to the starting Oligonucleotide but may reduce purity in some reactions (Dye Blubs may appear). Also sequence reactions in this region, depending on the sequence, sometimes show a bad performance.

Add 500 µl **Binding Buffer** to the completed cycle sequencing reaction (20 – 100 µl). Add 150 µl of **Isopropanol** to the mixture and mix very well by pipetting or vortexing. Transfer the sample completely onto a Spin Filter and centrifuge for 4 min at maximum speed.
In case of removal of blobs coming from contamination by primer dimers the addition of Isopropanol is not helpful and should be omitted.

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.

Special Protocol 3: Purification and concentration of PCR - products from 200 µl PCR reactions

Please read the instructions carefully and conduct the prepared procedure !

for MSB® Spin PCRapace and Invisorb® Fragment CleanUp

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

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer*** *– see instruction page: 14*

1. Binding of the PCR-fragments

For PCR-mixture 200 µl

Add **1000 µl Binding Buffer** to the PCR sample and mix very well by pipetting or vortexing. Transfer the sample in two aliquots onto a Spin Filter and centrifuge for 2 min at 11.000 x g (11.000 rpm).Remove the filtrate and centrifuge again for 3 minutes

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. Centrifugation 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 needed buffer volumes in protocol 1 and 2. The needed amount of protocol 3 is not considered.*

**** You can order additional Binding Buffer (Cat. No. 10202220) for this application by phone (++49 (0)30 948928910/ 01) or by mail).***

2. *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.*
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.*

Protocol 4: Extraction of a DNA-fragment from an agarose gel slice

Please read the instructions carefully and conduct the prepared procedure!

for Invisorb® Spin DNA Extraction Kit and Invisorb® Fragment CleanUp

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

Attention: *Please be aware, that you have to prepare the* ***Binding Enhancer*** *– see instruction page: 16*

1. Excise the DNA-fragment from the agarose gel with a sharp scalpel. Minimize the agarose gel slice. Check the weight.
For gel slices up to 150 mg add 500 µl Gel Solubilizer S.
For gel slices > 150 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. Incubation under continuous shaking (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 some times 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 minute. Discard the filtrate. For reaction volumes > 800 µl reload the residual 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 into a new 1.5 ml Receiver Tube.
Add at least 20 µl Elution Buffer directly onto the center of the Spin Filter.
Incubate at room temperature for 5 minutes. 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.

Protocol 5: Instruction for repair of A-overhangs in DNA fragments after gel purification

for Invisorb® Spin DNA Extraction Kit and Invisorb® Fragment CleanUp

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

Add 2 Units Standard Taq DNA Polymerase (no proofreading activity, article number 302030XX), 3.5 µl Taq Buffer, 0.6 µl dNTP's (10 mM each) $MgCl_2$.
The final concentration in the mixture must be 1.5 mM

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

After this treatment, the repaired fragment can be used in cloning/ligation experiments

Troubleshooting of the MSB® Spin PCRapace and Invisorb® Fragment CleanUp

Problem	Cause	Comments and suggestions
low recovery	poor elution of DNA	add the elution buffer directly onto the centre of the Spin Filter (even if a small elution volume is used).
	problems with mineral oil	apply the correct centrifugation steps Take a higher volume of **Binding Buffer** Wash once with **Binding Buffer**

Troubleshooting of the Invisorb® Spin DNA Extraction Kit and Invisorb® Fragment CleanUp

Problem	Cause	Comments and suggestions
low recovery	incorrect **Wash Buffer** or no ethanol added	prepare the **Wash Buffer** exactly as described in the manual. storage of **Wash Buffer** with firmly fixed cap.
	poor elution of DNA	add the **Elution Buffer** directly onto the centre of the Spin Filter (even if a small elution volume is used).
	TBE buffered gels are used	the binding of DNA fragments under TBE buffer condition is slightly reduced for smaller fragments than 500 bp please use TAE agarose gels
	ineffective solubilization of the agarose gel slice	the gel slice must be completely dissolved
	no **Binding Enhancer** added	add the amount of **Binding Enhancer** needed to the solubilized suspension.
problems with down stream application, e.g. ligation	contamination with salt components	Washing of the Spin Filters as described in the manual
	contamination with agarose traces	prolong the incubation time with **Wash Buffer** to 5 minutes before centrifugation.
	contamination of the final DNA with ethanol	wash the Spin Filter one time with **Gel Solubilizer S**. keep the given centrifugation time, extend it if necessary (test the smell)

Ordering information

Product	Package Size	Catalogue No.
MSB® Spin PCRapace	50 purifications	1020220200
MSB® Spin PCRapace	250 purifications	1020220300
MSB® Spin PCRapace	500 purifications	1020220400
Single components		
Elution Buffer	15 ml	1020224000
Invisorb® Spin DNA Extraction Kit	50 purifications	1020110200
Invisorb® Spin DNA Extraction Kit	250 purifications	1020110300
Single components		
Gel Solubilizer	30 ml	1020118000
Wash Buffer (add 42ml ethanol)	18 ml	1020113000
Elution Buffer	15 ml	1020114000
Invisorb® Fragment CleanUp	50 purifications	1020300200
Invisorb® Fragment CleanUp	250 purifications	1020300300
Single components		
Wash Buffer (add 42 ml ethanol)	18 ml	1020303000
Elution Buffer	15 ml	1020304000
Gel Solubilizer S	30 ml	1020308000
Related products		
Invisorb® DNA CleanUp	50 purifications	1020400200
Invisorb® DNA CleanUp	250 purifications	1020400300

Possible suppliers for Isopropanol

Carl Roth
2-Propanol
Rotipuran >99.7%, 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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