Instructions for use
MSB® Spin PCRapace

INVITEK diagnostics

Language: EN

REF 1020220300
1020220400

250 preprarations
500 preprarations

Invitek Molecular GmbH
Robert-Rössle-Straße 10
13125 Berlin
Germany

Instruction

MSB® Spin PCRapace

With only 7 min of hands on time, the **MSB® Spin PCRapace** is the fastest system for purification of up to 100 µl PCR products (80 bp - 30 kb) in low elution volume from dNTPs, primers, enzymes, additives and salts using the unique **MSB® technology** – a washing step is not required.
The kit can be used for the clean-up of DNA fragments as well as for removal of salts and enzymes from restrictions digestion, ligation and cDNA synthesis mixtures.
Further, the **MSB® Spin PCRapace** can be used for the concentration of PCR products and for the removal of dye terminators from DNA cycle sequencing reactions. The recovery of PCR product is 80 – 95 %.

Contents

Kit contents of the MSB® Spin PCRapace

	250 preps	500 preps
Catalogue No.	1020220300	1020220400
Binding Buffer	63 ml (final volume 163 ml)	2 x 63 ml (final volume 2 x 163 ml)
Elution Buffer	30 ml	60 ml
Spin Filter	5 x 50	10 x 50
2.0 ml Receiver Tubes	5 x 50	10 x 50
1.5 ml Receiver Tubes	5 x 50	10 x 50
Manual	1	1
Initial steps	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.	add 100 ml 99.7% Isopropanol to each bottle **Binding Buffer**, mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.

Symbols


Manufacturer

Lot number

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

Catalogue number


Expiry date


Consult operating instructions


Temperature limitation


Do not reuse


Humidity limitation

Storage

All buffers and kit contents of the **MSB® Spin PCRapace** should be stored at room temperature, buffer stability is guaranteed for 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 **MSB® Spin PCRapace** 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 **MSB® Spin PCRapace** has been tested against predetermined specifications.

If you have any questions or problems regarding any aspects of **MSB® Spin PCRapace** 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.

For technical support or further information, please contact:
Email: techsupport@invitek.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 MSB® Spin PCRapace").

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. Single stranded DNA may diverge. The maximum length of primers witch can be removed is 40 bp. 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.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 **MSB® Spin PCRapace** 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 phases for the components of the **MSB® Spin PCRapace** are listed as follows:

None

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 the membrane adsorption-based purification of PCR products as well the purification of DNA fragments from enzymatic reaction mixtures using the high-performance **MSB® technology**.

	MSB® Spin PCRapace
Sample Volume	up to 100 µl
Recovery	80 – 95 %
Binding capacity	10 µg
Elution Volume (minimal)	10 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

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 each kit are optimized for efficient recovery of DNA and removal of contaminants like salts, enzymes, nucleotides, 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 on the following pages.

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 **MSB® kit** has been designed for 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® system is much more concentrated than DNA purified with 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 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. 7 min

The **MSB® Spin PCRapace** is specially designed for ultra-fast and efficient direct purification of approx. 100 µl PCR products from 80 bp up to 30 kb from amplification reactions.
With max. 7 min of hands on time the **MSB® Spin PCRapace** is the fastest system for the separation of PCR products from dNTPs, primers, additives, labelling reagents (biotin, radioactive ATP etc.) and salts. Also all enzymes are removed, independent of size and secondary structure. The recovery of PCR product is 80 – 95 %. The kit is also useful for cleanup of DNA fragments from:

- restriction digestion mixture
- dephosphorylation
- primed synthesis
- endlabeling
- nick translatation
- ligation mixture
- cDNA synthesis mixtures

Additionally, the kit can be used for:

- concentration of DNA fragments
- purification of linearized pDNA from restriction mixtures
- 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 10 µl low salt buffer or Water.

The purified PCR product can be used in subsequent downstream applications:

- Sequencing
- Labeling experiments
- Hybridization
- Transcription
- Digestion with restriction enzymes
- Amplification
- Ligation and transformation

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 “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 recommended.
- 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 supplied by user

- Microcentrifuge (≥ 11.100 x g)
- Pipettes and filter tips
- 1.5 ml and 2.0 ml reaction rubes
- Isopropanol (99.7%)

*The **MSB® Spin PCRapace** 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
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 like enzyme buffer, enzyme, primers and nucleotides during the binding step
- Elution of highly pure DNA fragment or PCR product

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.

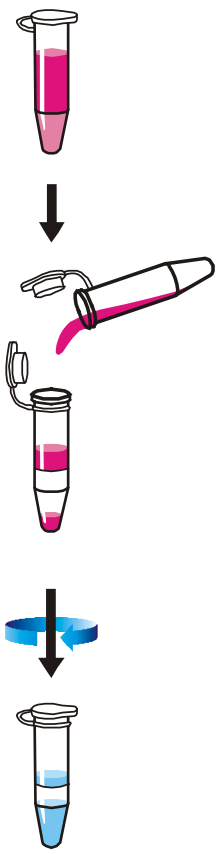
Elution of PCR products or DNA fragments

DNA is eluted from the column using 10 - 50 µl Elution Buffer.
Eluting twice with 30 - 50 µl each 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.

Preparing reagents and buffers of the MSB® Spin PCRapace

250 preps
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
500 preps
Add 100 ml 99.7% Isopropanol to each bottle **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

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: 9*

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, 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. 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 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: 9*

1. Binding of the (fluorescent) labeled DNA

Add **500 µl Binding Buffer** to the completed cycle sequencing reaction (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.

Additional Protocol 3: Purification and concentration 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: 9*

1. Binding of the PCR-fragments

For PCR-mixture 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. 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 required buffer volumes in protocol 1 and 2. The amount needed for protocol 3 is not considered.*
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.*

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.
MSB® Spin PCRapace	250 purifications	1020220300
MSB® Spin PCRapace	500 purifications	1020220400
InviSorb® Fragment CleanUp	250 purifications	1020300300

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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INVITEK
diagnostics

PORTUGAL
Zona Industrial de Tondela, ZIM II, Lote 2 e 6
63460-070 Tondela
Portugal

Phone: +351 232 817 817

GERMANY
Robert-Rössle-Str. 10
13125 Berlin
Germany

Phone: +49 30 9489 2908

info@invitek.com
www.invitek.com

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