

Instruction for Invisorb® DNA CleanUp

The **Invisorb® DNA CleanUp** provides a convenient tool for fast and efficient direct purification of contaminated DNA after DNA isolation as well as for fast and efficient direct purification of PCR* products from 80 bp up to 30 kb from amplification reactions

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

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

Invisorb® is a registered trademark of STRATEC Molecular GmbH.

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

© 2011 STRATEC Molecular, all rights reserved.

Contents

Kit contents of Invisorb® DNA CleanUp	3
Reagents and equipment to be supplied by user	3
Symbols	4
Storage	4
Quality control	4
Intended use	5
Product use limitation	5
Safety information	5
Product characteristic of Invisorb® DNA CleanUp	6
Principle and procedure	7
Important notes	7
Important points before starting a protocol	7
Preparing reagents and buffers	8
Scheme	9
Instructions	10
<i>Protocol 1:</i> Purification of contaminated DNA after DNA isolation from up to 50 µl	10
<i>Protocol 2:</i> Purification of contaminated DNA after DNA isolation up to 50 - 200 µl	11
<i>Protocol 3:</i> Purification of PCR-products from PCR reaction mixes up to 50 µl	12
<i>Protocol 4:</i> Purification of PCR-products from PCR reaction mixes up to 50 - 200µl	13
Troubleshooting	14
Appendix	14
General notes on handling DNA	14
Order information	14

Kit contents of Invisorb[®] DNA CleanUp









Store all kit components at room temperature !

	3 purification	10 purifications	50 purifications	250 purifications
Catalog No.	1020400100	1020400900	1020400200	1020400300
Buffer P	2 ml	2 x 2 ml	20 ml	80 ml
Wash Buffer	5 ml (ready to use)	10 ml (ready to use)	24 ml (final volume 60 ml)	1 x 80 ml (final volume 200 ml)
Wash Buffer I	15 ml (ready to use)	15 ml (ready to use)	30 ml (final volume 60 ml)	80 ml (final volume 160 ml)
Wash Buffer II	15 ml (ready to use)	15 ml (ready to use)	18 ml (final volume 60 ml)	60 ml (final volume 200 ml)
Elution Buffer	2 ml	2 ml	15 ml	30 ml
Spin Filter	3	10	50	250
2.0 ml Receiver Tubes	3	10	50	250
1.5 ml Receiver Tubes	3	10	50	250
Manual	1	1	1	1
Initial steps			add 36 ml 96-100% ethanol to the bottle Wash Buffer add 30 ml 96-100% ethanol to the bottle Wash Buffer I add 42 ml 96-100% ethanol to the bottle Wash Buffer II	add 120 ml 96-100% ethanol to each bottle Wash Buffer add 80 ml 96-100% ethanol to the bottle Wash Buffer I add 140 ml 96-100% ethanol to the bottle Wash Buffer II

Reagents and equipment to be supplied by user

- microcentrifuge
- measuring cylinder (250 ml)
- pipette and pipette tips
- disposable gloves
- reaction tubes (1.5 ml or 2.0 ml)
- vortexer
- 96-100% ethanol

Symbols

	manufacturer
	lot number
	catalogue number
	date of manufacture
	expiry date
	consult operating instructions
	temperature limitation
	do not reuse

Storage

The **Invisorb® DNA CleanUp** should be stored dry, at room temperature and is stable for at least 12 months under these conditions. Make sure that all components have room temperature. If there should be any precipitates in the reagents provided, dissolve them by careful warming up to 37°C. **Room temperature (RT) is defined as range from 15 - 30°C.**

Quality control

STRATEC Molecular guarantees the correct function of the **Invisorb® DNA CleanUp** for applications as described in the manual. In accordance with STRATEC Molecular's certified Quality Management System each component of the **Invisorb® DNA CleanUp** were tested against predetermined specifications to ensure consistent product quality.

All products sold by STRATEC Molecular are subjected to extensive quality control procedures according to ISO 9001-2000 and are warranted to perform as described when used correctly. Any problems should be reported immediately.

If you have any questions or problems regarding any aspects of **Invisorb® DNA CleanUp** or other STRATEC Molecular products, please do not hesitate to contact us.

For technical support or further information please contact:

from Germany +49-(0)30-9489-2901/ 2910

from abroad +49-(0)30-9489-2907

or contact your local distributor.

Intended use

The **Invisorb® DNA CleanUp** provides a convenient tool for fast and efficient cleanup of strong contaminated DNA, DNA fragments and PCR products, DNA isolated using classical procedures and DNA from reaction mixtures like the bisulfite method used in methylation analysis.

DNA purified by the **Invisorb® DNA CleanUp** is ready to use for a broad panel of downstream applications.

The product is intended for use by professional users such as technicians, physicians and biologists trained in molecular biological techniques. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modification of DNA followed by signal detection or amplification.

Product use limitation

The Kit is neither for isolation and purification of pDNA, nor for isolation and purification of RNA. The included chemicals and spin filter are only useable once.

When differing the starting material or the protocol, no guarantee in operability is issued.

The **Invisorb® DNA CleanUp** is developed, designed, and sold for research purposes only. It is not to be used for human diagnostic or drug purposes or to be administrated to humans unless expressly cleared for that purpose by the Food and Drug Administration.

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

The kit contents are unfit for consumption.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. Heed the legal requirements for working with biological material! For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.invitek.de for each STRATEC Molecular kit and contained components.

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries.

Below are listed European Community risk and safety phrases for the components of the **Invisorb® DNA CleanUp** to which they apply.

Buffer P



danger

H 225-319-336 P210-233-305-351-338

H225:	Highly flammable liquid and vapour.
H319:	Causes serious eye irritation.
H336:	May cause drowsiness or dizziness.
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.
P210:	Keep away from heat/sparks/open flames/hot surfaces. — No smoking.
P233:	Keep container tightly closed.
P305+P351+P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P273:	Avoid release to the environment.

Wash Buffer I



warning

H 302-312-332-412 EUH032 P273

Emergency medical information in English and German can be obtained 24 hours a day from:

Poison Information Center Freiburg, Germany: Phone.: +49 (0)761-19240

Product characteristic of Invisorb® DNA CleanUp

Starting material	Rate of recovery	Time for preparation
up to 200 µl of DNA eluate up to 100 µl of amplification reaction volume up to 100 µl of reaction mixtures	60 % - 85 % depends on fragment length	less than 10 minutes

The **Invisorb® DNA CleanUp** provides a convenient tool for fast and efficient cleanup of strong contaminated DNA, DNA fragments and PCR products (80 bp up to 30 kb), DNA isolated using classical procedures and DNA from reaction mixtures like the bisulfite method used in methylation analysis.

The optimized Binding Buffer adjust the condition and DNA or DNA-fragments will be bound directly onto the surface of a spin filter column during contaminants will be passed through during washing step(s).

The buffer volumes are balanced to minimize pipetting steps. The **Buffer P** is added directly to the sample, adjusting the binding condition for DNA or DNA-fragments and the mixture is applied to the spin filter column. The DNA fragments are bound directly onto the membrane of a spin filter column. After washing to remove contaminants the DNA or DNA fragments are eluted in a low-salt buffer or ddH₂O.

The isolation protocol as well as all buffers are optimized to provide high yield and purity of the recovered DNA fragments. The hands-on time necessary for the whole procedure is reduced to a minimum. The purification process takes less than 10 minutes.

The purification protocol as well as all buffers are 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 purified DNA fragments are ready to use in various downstream application such as:

- Digestion with restriction enzymes
- Hybridization
- Labeling
- Cloning
- Sequencing
- *In vitro* Transcription

The PCR method is covered by U.S. Patents 4,683,195 and 4,683,202 owned by Hoffmann-LaRoche Inc. The purchase of the **Invisorb® DNA CleanUp** cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-LaRoche Inc.

Principle and procedure

The **Invisorb® DNA CleanUp** procedure comprises following steps:

1. adjustment of binding conditions
2. removal of contaminants and elimination of ethanol
3. elution of DNA

All steps are performed without use of phenol, chloroform, CsCl, ethidium bromide, and without alcohol precipitation.

This manual contains four protocols.

Adjustment of binding conditions

The **Buffer P** is simply added directly to the sample, adjusting the binding condition for DNA or DNA-fragments.

Binding of DNA or DNA fragments

The mixture is applied to an Invisorb® Spin column and the DNA or PCR products is adsorbed onto membrane while the contaminating RNA, proteins, metabolitesm PCR inhibitors or nucleotides, salts, primer, polymerase etc. remain in the lysate and are drawn through by centrifugal force.

Removing residual contaminants

Contaminations like endonucleases or others are efficiently washed away using the relevant **Wash Buffers**, while the DNA remains bound to the membrane.

Elution of DNA

DNA is eluted from the column using 50 - 100 µl Elution Buffer.

Eluting twice each with 30 - 100 µl leads to slightly recovery of DNA. By the use of small elution volumes DNA concentration can be raised. Elution volumes should not fall below 30 µl, otherwise the yield will be reduced. The eluted DNA is ready to use in different downstream applications.

Important notes

Important points before starting a protocol

After receiving the kit, check the kit components for damage. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety information" (page 6). Do not use damaged kit components, since their use may lead to poor kit performance.

- always change pipet tips between liquid transfer. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips
- all centrifugation steps are carried out at room temperature
- when working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles
- discard gloves if they become contaminated
- do not use kit components from other kits with the kit you are currently using, unless the lot numbers are identical
- avoid microbial contamination of the kit reagents

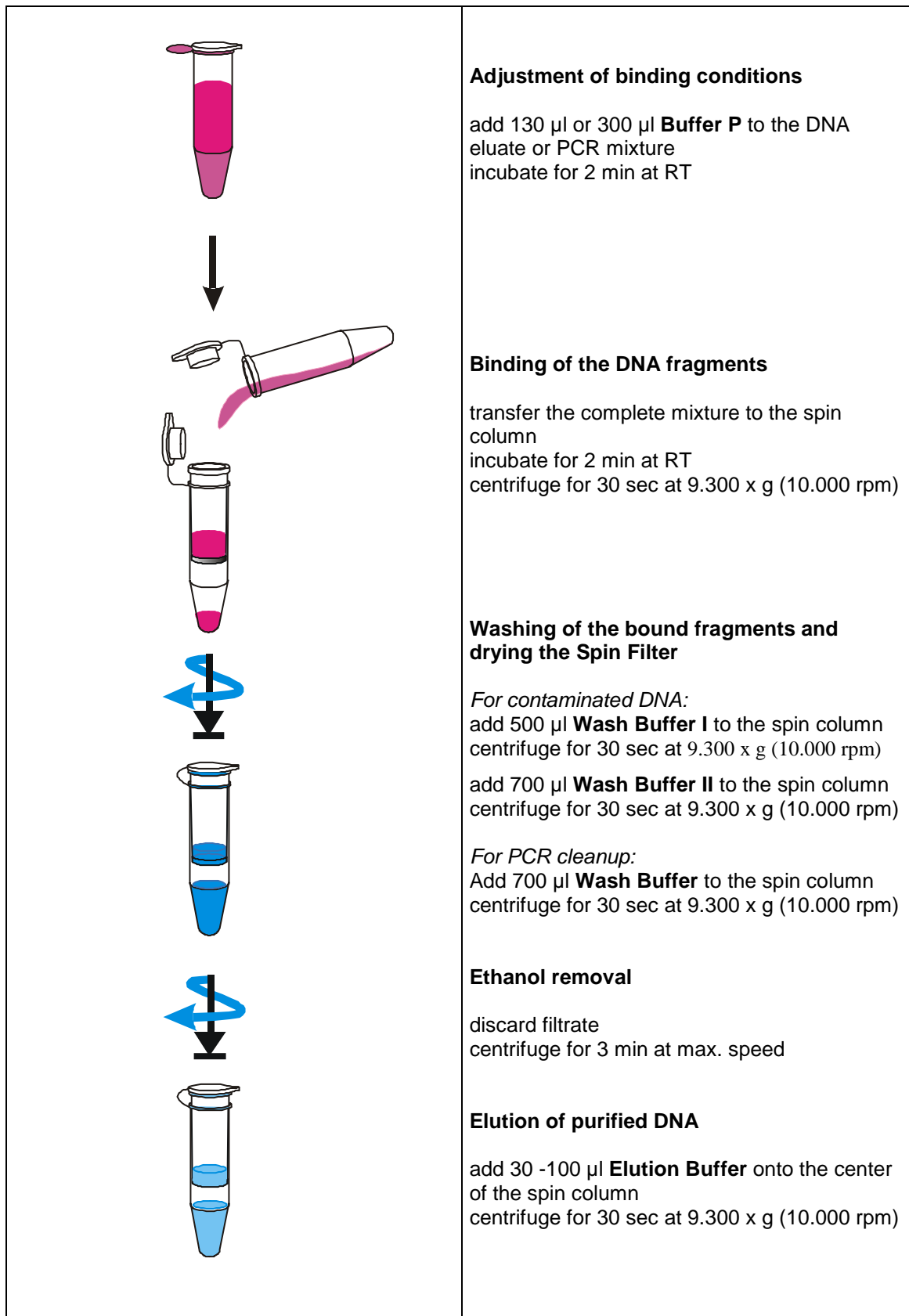
Preparing reagents and buffers

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 at our webpage : www.invitek.de)

1. Label the needed amount of 2.0 ml Receiver Tubes
2. Place spin filters into labeled 2.0 ml Receiver Tubes
3. Labeling the needed amount of 1.5 ml Receiver Tubes

3 DNA extractions:
Wash Buffers are ready to use !
10 DNA-extractions:
Wash Buffers are ready to use !
50 DNA-extractions:
Add 36 ml 96-100% ethanol to the bottle Wash Buffer Add 30 ml 96-100% ethanol to the bottle Wash Buffer I Add 42 ml 96-100% ethanol to the bottle Wash Buffer II
250 DNA-extractions:
Add 120 ml 96-100% ethanol to each bottle Wash Buffer Add 80 ml 96-100% ethanol to the bottle Wash Buffer I Add 140 ml 96-100% ethanol to the bottle Wash Buffer II

Scheme of the Invisorb® DNA CleanUp



Instructions

The following notes are valid for all protocols:

Note: *The centrifugation steps were made with the **Centrifuge 5415 D** from Eppendorf. The indicated **rpm amounts** are referring to this centrifuge.*

Protocol 1: Purification of contaminated* DNA after DNA isolation from up to 50 µl

Please read the instructions carefully and conduct the prepared procedure !

The following protocol is designed to purify modified and double-stranded contaminated DNA isolated by using classical procedures, like DNA after CTAB purification procedure, or DNA isolated from difficult starting materials like chocolate including PCR inhibitors, or DNA from reaction mixtures like bisulfite method used in methylation analysis resulting in high endconcentrations of DNA using centrifugation-driven sample processing. DNA fragments ranging from 80 bp to 30 kb

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

1. Add 130 µl **Buffer P** directly to the tube with the DNA eluate. Transfer the suspension completely into a Spin Filter. Incubation for 2 min at RT.
2. Centrifuge for 30 sec at 9.300 x g (10.000 rpm).
3. Add 500 µl **Wash Buffer I** to the Spin Filter and centrifuge for 30 sec at 10,000 rpm.
4. Add 700 µl **Wash Buffer II** to the Spin Filter and centrifuge for 30 sec at 10,000 rpm.
5. Discard the filtrate. Remove the residual ethanol of the **Wash Buffer II** by centrifugation for 3 min at maximum speed 13.400 – 16.500 x g (12.000 – 14.000 rpm).
6. Transfer the Spin Filter into a new 1.5 ml Receiver Tube.
Add at least 30 µl **Elution Buffer** directly onto the center of the Spin Filter.
Incubate at room temperature for 3 minutes. Centrifuge for 1 minute at 9.300 x g (10.000 rpm)

Note: *To increase the final DNA yield we recommend to use 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.
A prewarming of the **Elution Buffer** to 70°C increase also the recovery.*

Protocol 2: Purification of contaminated DNA after DNA isolation from up 50-200 µl

Please read the instructions carefully and conduct the prepared procedure !

The following protocol is designed to purify modified and double-stranded DNA from using classical procedures and DNA from reaction mixtures like DNA after CTAB purification procedure, or DNA isolated from difficult starting materials like chocolate, or DNA from reaction mixtures like the bisulfite method used in methylation analysis resulting in high endconcentrations of DNA . Fragments ranging from 80 bp to 30 kb are purified from primers, nucleotides, polymerases and salts using centrifugation-driven sample processing.

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

1. Mix 300 µl **Buffer P** with the DNA eluate in a 1.5 ml reaction tube. Transfer the suspension completely into a Spin Filter. Incubation for 2 min.
2. Centrifuge for 30 sec at 9.300 x g (10.000 rpm).
3. Add 500 µl **Wash Buffer I** to the Spin Filter and centrifuge for 30 sec at 9.300 x g (10.000 rpm).
4. Add 700 µl **Wash Buffer II** to the Spin Filter and centrifuge for 30 sec at 9.300 x g (10.000 rpm).
5. Discard the filtrate. Remove the residual ethanol of the **Wash Buffer II** by centrifugation for 3 min at maximum speed 13.400 – 16.500 x g (12.000 – 14.000 rpm).
6. Transfer the Spin Filter into a new 1.5 ml Receiver Tube.
Add at least 30 µl **Elution Buffer** directly onto the center of the Spin Filter.
Incubate at room temperature for 3 min.
7. Centrifuge for 1 minute at 9.300 x g (10.000 rpm).

Note: *To increase the final DNA yield we recommend to use 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.
A longer incubation time with **Elution Buffer** (up to 10 minutes) leads also to a slightly higher final yield.
A prewarming of the **Elution Buffer** to 70°C increase also the recovery*

Protocol 3: Purification of PCR-products from PCR reaction mixes up to 50 µl

Please read the instructions carefully and conduct the prepared procedure !

The following protocol is designed to purify double-stranded DNA fragments from PCR reactions and other enzymatic reactions e.g., restriction digestion resulting in high end-concentrations of DNA Fragments ranging from 80 bp to 30 kb are purified from enzymes, primers, nucleotides, polymerases and salts using the Invisorb Spin columns in a microcentrifuge. The DNA-binding capacity of the Invisorb Spin columns is 5 µg.

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

1. Add 130 µl **Buffer P** directly to the PCR reaction tube (mineral oil overlaying does not disturb). Transfer the suspension completely into a Spin Filter.
Incubation for 1 min.
2. Centrifuge for 30 sec. at 9.300 x g (10.000 rpm).
3. Add 700 µl **Wash Buffer** to the Spin Filter and centrifuge for 30 sec at 9.300 x g (10.000 rpm).
4. Discard the filtrate. Remove the residual ethanol of the **Wash Buffer** by centrifugation for 3 min at maximum speed 13.400 – 16.500 x g (12.000 – 14.000 rpm).
5. Transfer the Spin Filter into a new 1.5 ml Receiver Tube.
Add at least 30 µl Elution Buffer directly onto the center of the Spin Filter.
Incubate at room temperature for 3 minutes. Centrifuge for 1 minute at 9.300 x g (10.000 rpm)

Note: *To increase the final DNA yield we recommend to use 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.
A prewarming of the **Elution Buffer** to 70°C increase also the recovery*

Protocol 4: Purification of PCR-products from PCR reaction mixtures from 50 µl - 200 µl

Please read the instructions carefully and conduct the prepared procedure !

The following protocol is designed to purify double-stranded DNA fragments from PCR reactions and other enzymatic reactions e.g., restriction digestion resulting in high end-concentrations of DNA Fragments ranging from 80 bp to 30 kb are purified from enzymes, primers, nucleotides, polymerases and salts using the Invisorb Spin columns in a microcentrifuge. The DNA-binding capacity of the Invisorb Spin columns is 5 µg.

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

1. Mix 300 µl **Buffer P** with the PCR reaction mixture in a 1.5 ml reaction tube (mineral oil overlaying does not disturb). Transfer the suspension completely into a Spin Filter. Incubation for 1 min.
2. Centrifuge for 30 sec at 9.300 x g (10.000 rpm).
3. Add 700 µl **Wash Buffer** to the Spin Filter and centrifuge for 30 sec at 9.300 x g (10.000 rpm).
4. Discard the filtrate. Remove the residual ethanol of the **Wash Buffer** by centrifugation for 3 min at maximum speed (12,000 – 14,000 rpm).
5. Transfer the Spin Filter into a new 1.5 ml Receiver Tube. Add at least 30 µl **Elution Buffer** directly onto the center of the Spin Filter. Incubate at room temperature for 3 min.
6. Centrifuge for 1 minute at 9.300 x g (10.000 rpm).

Note: *To increase the final DNA yield we recommend to use 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. A longer incubation time with Elution Buffer (up to 10 minutes) leads also to a slightly higher final yield. A prewarming of the **Elution Buffer** to 70°C increase also the recovery*

Note: *For purification of PCR-fragments from larger amounts of PCR reaction mixtures, please call for a special protocol.*

Troubleshooting

Problem/ probable cause	Comments and suggestions
<p>low recovery</p> <p>incorrect Wash Buffer/ Wash Buffer I or II or no ethanol added</p> <p>poor elution of DNA</p>	<p>prepare the Wash Buffer / Wash Buffer I or II exactly as described in the manual</p> <p>storage of Wash Buffer with firmly fixed cap</p> <p>add the Elution Buffer directly onto the center of the Spin Filter (even if a small elution volume is used)</p>
<p>problems with down stream application e.g. ligation</p> <p>contamination with salt components</p> <p>contamination of the final DNA with ethanol</p>	<p>washing of the Spin Filters as described in the manual. Prolong the incubation time with Wash Buffers to 5 minutes before centrifugation.</p> <p>keep the given centrifugation time, extend it if necessary (test the smell)</p>

Appendix

General notes on handling DNA

Nature of DNA

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

Storage of DNA

Store DNA and other small circular DNAs at +2 to +8°C. Storing pDNA at -15 to -25°C can cause shearing of DNA, particularly if the DNA is exposed to repeated freeze-thaw cycles.

Drying, dissolving and pipetting DNA

Avoid overdrying DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution. DNA and other small circular DNAs can be vacuum-dried.

To help dissolve the DNA, carefully invert the tubes several times after adding buffer and tap the tube gently on the side. Alternatively let the DNA stand in buffer overnight at +2 to +8°C. Minimize vortexing of DNA since this can cause shearing.

Order information

Product	Package Size	Order No.
Invisorb® DNA CleanUp	3 preparations	1020400100
Invisorb® DNA CleanUp	10 preparations	1020400900
Invisorb® DNA CleanUp	50 preparations	1020400200
Invisorb® DNA CleanUp	250 preparations	1020400300