ISOLATE II
PCR and Gel Kit

Product Manual

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ISOLATE II PCR and Gel Kit

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1. KIT CONTENTS

COMPONENT	10 Preps	50 Preps	250 Preps
ISOLATE II PCR and Gel Columns (yellow)	10	50	250
Collection Tubes (2 mL)	10	50	250
Binding Buffer CB	10 mL	40 mL	200 mL
Wash Buffer CW† (concentrate)	6 mL	25 mL	2 x 50 mL
Elution Buffer C	13 mL	13 mL	30 mL
Bench Protocol Sheet	1	1	1

† Before use, add indicated volume of 96-100% ethanol and mark wash buffer bottle label.

2. DESCRIPTION

The ISOLATE II PCR and Gel Kit is a simple, reliable and fast method for the isolation of high-quality DNA fragments from enzymatic reactions, such as PCR, as well as from agarose gels. It uses a binding buffer and collection tube for silica-membrane-based binding of DNA fragments, in the presence of chaotropic salt. Contaminants are removed by simple washing steps and pure DNA is eluted under low salt conditions. The isolated DNA is suitable for use with all downstream molecular biology applications. Please read this manual carefully to familiarise yourself with the ISOLATE II PCR and Gel protocol before starting (also available on www.bioline.com). More experienced users can refer to the bench-top protocol for quick referencing during the procedure.

3. STORAGE

All kit components should be stored at room temperature (18 - 25°C) and are stable for up to 1 year.

4. SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, gloves and safety glasses.

Binding Buffer CB contains chaotropic salt.

For detailed information, please consult the material data safety sheets (MSDSs) available on our website at www.bioline.com.

5. PRODUCT SPECIFICATIONS

The ISOLATE II PCR and Gel Kit is specially designed for the rapid and efficient isolation of extremely pure DNA from enzymatic reactions, as well as TAE or TBE agarose gels. The kit offers very high DNA binding capacity of up to 25 µg and ensures complete removal of contaminants such as nucleotides, primers, enzymes, mineral oil, PCR additives (e.g. salts, betaine, DMSO), commonly used detergents (Tween 20 or Triton X-100), dyes (e.g. ethidium bromide, crystal violet) and unbound labels or tags.

ISOLATE II PCR AND GEL KIT SPECIFICATIONS

Binding capacity	25 µg
Sample material	Up to 200 µL of PCR product or 200 mg of gel
Optimal recovery	< 15 µg 100 - 500 bp 30 µL
Elution volume	15 - 30 µL
Fragment length	50 bp to ≥ 20 kb

PCR primers from reactions are eliminated while small DNA fragments are still bound and purified with high recovery. The cut-off for small DNA fragments can be shifted from < 50 bp to several hundred base pairs by diluting Binding Buffer CB to remove primer-dimers from target PCR products.

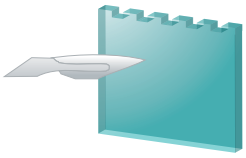
A yellow pH indicator in Binding Buffer CB ensures optimal binding conditions and makes it easier to identify undissolved agarose during DNA gel extraction.

The preparation time is 10 min for 6 PCR purifications, or 20 min for 6 gel extractions.

The purified DNA is suitable for applications such as hybridization, sequencing, PCR, restriction, digestion, ligation, *in vitro* transcription, labeling or many kinds of enzymatic reactions.

PCR and Gel Clean-up

PCR Clean-Up
Add 200 µL Binding Buffer CB per 100 µL of PCR product
Mix



Gel extraction
Excise DNA fragment

Gel solubilization
Add 200 µL Binding Buffer CB per 100 mg gel
Incubate 50°C, 5 - 10 min and vortex

Bind DNA
Load sample

11,000 x g, 30s

Wash silica membrane
1st wash 700 µL Wash Buffer CW
2nd wash 700 µL Wash Buffer CW (recommended)

1st and 2nd
11,000 x g, 30s

Dry silica membrane
11,000 x g, 1 min

Elute DNA
Add 15 - 30 µL Elution Buffer C
Incubate RT, 1 min

11,000 x g, 1 min

Isolated DNA

6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY THE USER

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

- 96-100% ethanol†
- Microcentrifuge tubes (1.5 mL)
- Sterile DNase-free tips
- Pipettes
- Microcentrifuge (capable of 11,000 x g)
- Vortex mixer
- Thermal heating block
- Scalpel to cut agarose gels
- Diffusion buffer (500 mM ammonium acetate, pH 8.0, 0.1% SDS, 1 mM EDTA, 10 mM magnesium acetate) if extracting DNA from polyacrylamide gels

† *Molecular biology grade ethanol is recommended. Do not use denatured alcohol which contains unwanted additives such as methanol and acetone.*

7. IMPORTANT NOTES

7.1 REMOVAL OF SMALL DNA FRAGMENTS AND PRIMER-DIMERS

Removal of double stranded DNA > 50 bp can be achieved by diluting an aliquot of Binding Buffer CB with sterile water in an appropriate ratio and then proceeding with the standard PCR clean-up protocol. Diluting Binding Buffer CB lowers the binding efficiency for small fragments without compromising the recovery of larger PCR products; however, the dilution ratio will depend on the fragment size as well as detergents and additives. In general, if a PCR buffer system without special additives is used, adding 3 to 5 volumes of water to 1 volume of Binding Buffer CB will lead to removal of small fragments up to 100 bp, otherwise adding 1 to 3 volumes of water to 1 volume of Binding Buffer CB will be sufficient.

7.2 PH INDICATOR

The Binding Buffer CB is sufficiently buffered to maintain an optimal pH of 5.0 - 6.0. It will even bind small DNA fragments to the silica membrane of the ISOLATE II PCR and Gel Columns, for all standard PCR reaction buffers or agarose gel buffer systems. In addition, the colored binding buffer helps identify undissolved agarose during DNA gel extraction. A yellow color indicates the optimal pH < 6.0. If the pH increases to about 7.0 after adding the sample, the solution will turn green and above 7.0 the solution turns blue. If a change in color is observed, the pH should be corrected by adding more Binding Buffer CB or by titrating the pH back to < 6.0 with 4 M sodium acetate (pH 5.0) or small amounts of hydrochloric acid (HCl).

7.3 FACTORS AFFECTING DNA RECOVERY

DNA fragment sizeUpon completion of the wash steps with Wash Buffer CW, the DNA will adhere to the silica membrane. The standard elution buffer volume is 15 - 30 µL which is best for high DNA recovery and high DNA concentration for fragments < 1000 bp.

Large DNA fragments bind much more tightly and are much more difficult to elute than small DNA fragments. The ISOLATE II PCR and Gel Kit is recommended for DNA up to 10 - 15 kb. Longer fragments can be purified but recovery may be low and above 20 kb they may be damaged by centrifugation (mechanical shearing through the membrane).

Agarose gel extraction

Although TBE agarose gels can be used with the ISOLATE II PCR and Gel Kit, TAE is preferred as it gives better resolution (especially when run at a lower voltage and only long enough to separate the band of interest) and does not interact with the agarose, resulting in higher DNA yields.

When extracting the gel slice, exposure to UV light should be minimized and only up to 200 mg of gel per 400 µL of Binding Buffer CB used. Virtually unlimited amounts of gel can however, be loaded without clogging the column, by increasing Binding Buffer CB proportionally and using multiple loading steps.

If higher DNA concentrations are required, elute in less than 30 µL (although concentration can be more than doubled, total DNA recovery will be significantly reduced for volumes less than 15 µL).

7.4 SALT CARRY-OVER

Salt carry-over into the eluate always happens with chaotropic salt. Although this can only be minimized by extensive washing, this is unnecessary, since the final concentration of chaotropic salt in eluates is far too small to have any negative effect.

7.5 BUFFER PREPARATION AND PARAMETERS

Preparing Wash Buffer CW

Add 96 - 100% ethanol (not supplied) to Wash Buffer CW Concentrate: 24 mL for the 10 prep kit, 100 mL for the 50 prep kit and 200 mL x 2 for the 250 prep kit.

Elution buffer

We highly recommended eluting DNA with Elution Buffer C (pH 8.5), which is provided with the kit. A standard TE buffer may also be used, however the EDTA in the buffer may cause problems in subsequent enzymatic reactions. Do not use deionized water since its pH is usually too acidic, instead dilute Elution Buffer C with distilled water and make sure the pH is still > 7.0. Unbuffered elution buffer should not be used.

Elution after gel extraction is 10 - 20% less efficient than elution of purified PCR products. In addition, elution of several kb long DNA fragments is 10 - 30% less efficient than elution of 500 bp fragments. To improve the DNA recovery after gel extraction, or for large DNA fragments, the following modifications can be applied to the standard elution procedure:

- Heat Elution Buffer C to 70°C and incubate on the column at 70°C for 5 min.
- Apply Elution Buffer C to the column and centrifuge at 30 - 50 x g for 1 min and then at 11,000 x g for 1 min.
- Perform up to 3 elution steps with 20 or 30 µL fresh Elution Buffer C.

8. PROTOCOLS

8.1 PCR CLEAN-UP

Suitable for PCR clean-up, DNA concentration and removal of salts, primers, enzymes, detergents, dyes, additives and other contaminants (see section 5).

Before you start:

- Make sure Wash Buffer CW is prepared (see section 7.5).

1 Sample preparation

For volumes < 30 µL, adjust the volume to 50 - 100 µL with water (removal of mineral oil is unnecessary).

Mix 1 volume of sample with 2 volumes of Binding Buffer CB.

Note: *For removal of small fragments like primer-dimers, dilutions of Binding Buffer CB can be used instead of 100% Binding Buffer CB. See section 7.1.*

2 Bind DNA

For each preparation, place one ISOLATE II PCR and Gel Column in a Collection Tube (2 mL) and load sample.

Centrifuge for 30s at 11,000 x g and discard flow-through. Reuse the Collection Tube for step 3.

3 Wash silica membrane

Add 700 µL Wash Buffer CW to the ISOLATE II PCR and Gel Column and centrifuge for 30s at 11,000 x g. Discard the flow-through and place the column back into the collection tube.

Recommended: *Repeat washing step to minimize chaotropic salt carry-over.*

4 Dry silica membrane

Centrifuge for 1 min at 11,000 x g, to remove residual ethanol. Place the ISOLATE II PCR and Gel Column in a 1.5 mL microcentrifuge tube (not supplied).

Note: *Residual ethanol might inhibit enzymatic reactions. Total removal of ethanol can be achieved by incubating the columns at 70°C for 2 - 5 min prior to elution.*

5 Elute DNA

Add 15 - 30 µL Elution Buffer C directly onto the silica membrane. Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g.

Note: *For alternative elution procedures see section 7.3.*

8.2 DNA EXTRACTION FROM AGAROSE GELS

Before you start:

- Make sure Wash Buffer CW is prepared (see section 7.5).

1 Excise and dissolve gel slice

Using a clean scalpel excise the DNA fragment from the gel. Remove excess agarose, determine the weight of the slice and transfer into a clean tube.

Add 200 µL Binding Buffer CB per 100 mg of 2% agarose gel*.

** For gels containing > 2% agarose, double the volume of Binding Buffer CB.*

Incubate sample at 50°C for 5 - 10 min, vortexing the sample briefly every 2 - 3 min until the gel slice is completely dissolved.

2 Bind DNA

Proceed with step 2 of the PCR clean-up protocol (see section 8.1).

8.3 DNA EXTRACTION FROM POLYACRYLAMIDE GELS

Polyacrylamide gels cannot be dissolved like agarose gels to extract the trapped DNA, instead the gel is crushed and soaked in a Diffusion buffer (not supplied).

Before you start:

- Make sure Diffusion buffer is prepared (see section 6).

1 Preparation of gel

Using a clean scalpel excise the DNA fragment from the gel. Remove as much excess polyacrylamide as possible and transfer into a clean 1.5 mL microcentrifuge tube (not supplied).

Crush the gel slice using a heat sealed disposable pipette tip to act as a pestle. The smaller the pieces, the better the DNA recovery.

2 Extract DNA

Add 200 µL of Diffusion Buffer to each 100 mg of crushed gel. Ensure all gel pieces are submerged and incubate for 30 - 60 min at 50°C or overnight at 37°C.

3 Remove polyacrylamide

Transfer to an ISOLATE II PCR and Gel Column and centrifuge for 1 min at 11,000 x g. Keep flow-through containing the DNA

Optional: *Steps 2 and 3 can be repeated to increase yield.*

4 Sample preparation

Mix 1 volume of sample with 2 volumes of Binding Buffer CB.

Note: *For removal of small fragments like primer-dimers dilutions of Binding Buffer CB can be used instead of 100% Binding Buffer CB. See section 7.1.*

Small amounts of precipitated SDS does not affect the purification and can be ignored.

5 Bind DNA

Proceed with step 2 of the PCR clean-up protocol (see section 8.1).

9. TROUBLESHOOTING GUIDE

INCOMPLETELY DISSOLVED GEL SLICE

POSSIBLE CAUSE	RECOMMENDED SOLUTION
Insufficient time and temperature	Check incubation temperature and volume of Binding Buffer CB. Increase incubation time, vortex every 2 min and check the integrity of the gel slice. Very large gel slices can be crushed before addition of Binding Buffer CB to shorten the melting time.

APPEARANCE OF ADDITIONAL BANDS ON AGAROSE GEL AFTER GEL EXTRACTION

POSSIBLE CAUSE	RECOMMENDED SOLUTION
DNA denatured during purification	If water is used for elution or agarose with low ion content is used for agarose gel electrophoresis, the formation of denaturated (single-stranded) DNA might be promoted. To re-anneal the DNA, simply add all components of the subsequent enzymatic reaction omitting the enzyme. Incubate at 95°C for 2 min and let the mixture cool slowly to room temperature. Add the enzyme and continue with downstream application. Use fresh running buffer and run at low voltage to lower the temperature. High temperatures can promote DNA denaturation during electrophoresis.

LOW DNA YIELD

POSSIBLE CAUSE	RECOMMENDED SOLUTION
Incompletely dissolved gel slice	Increase time or add another two volumes of Binding Buffer CB and vortex the tube every 2 min during incubation at 50°C.
Insufficiently dried silica membrane	Centrifuge 5 min at 11,000 x g or incubate column for 2 - 5 min at 70°C before elution to remove ethanol completely. Remove the spin column carefully from the Collection Tube and avoid contact of spin column with flow-through.
Incomplete elution	Particularly for larger DNA amounts (> 5 µg), long DNA fragments (> 1 kb), or after gel extraction, perform multiple elution steps with fresh buffer, heat to 70°C and incubate for 5 min, (See section 7.3) .
Reagents not prepared properly	Ensure Wash Buffer CW Concentrate is prepared with the correct volume of 96 - 100% ethanol and mix well before use (see section 7.5).

SUBOPTIMAL PERFORMANCE OF DNA IN SPECTROPHOTOMETER OR BIOANALYZER

POSSIBLE CAUSE	RECOMMENDED SOLUTION
Carry-over of residual silica particles	Spectrophotometers (e.g. NanoDrop®) are particularly sensitive to any particles included in the sample material. To pellet the silica particles, centrifuge > 2 min at 11,000 x g and keep supernatant for further use.

SUBOPTIMAL PERFORMANCE OF DNA IN SEQUENCING, RESTRICTION, OR LIGATION REACTIONS

POSSIBLE CAUSE	RECOMMENDED SOLUTION
DNA damaged by UV light	Reduce UV exposure time when excising a DNA fragment from an agarose gel.
Carry-over of ethanol	Before elution, centrifuge 5 min at 11,000 x g or incubate column for 5 - 10 min at 70°C to remove ethanol completely. Ethanol contamination is also indicated by gel loading problems (samples float out of gel slots). Remove the spin column carefully from the Collection Tube and avoid contact of the spin column with the flow-through. Use either a different brand of ethanol to reconstitute Wash Buffer CW or ethanol that is not denatured. The denaturing components may not evaporate as fast as ethanol and end up concentrated in the eluate, inhibiting enzymes like ligase.
Carry-over of chaotropic salts	Perform the optional washing step. Alternatively more Wash Buffer CW can be loaded before the drying step. (Note: This will reduce the number of preparations that you can do with the kit).
Elution of DNA with buffers other than Buffer C e.g. TE buffer (Tris/EDTA)	EDTA might inhibit sequencing reactions. In this case it is recommended to re-purify DNA and elute in Elution Buffer C.
Insufficient DNA template used for sequencing reaction	Quantify DNA by agarose gel electrophoresis before setting up sequencing reactions.

A. TECHNICAL SUPPORT

For technical assistance or more information on these products, please email us at tech@bioline.com

B. ORDERING INFORMATION

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II PCR and Gel Kit	10 Preps	BIO-52058
ISOLATE II PCR and Gel Kit	50 Preps	BIO-52059
ISOLATE II PCR and Gel Kit	250 Preps	BIO-52060

C. ASSOCIATED PRODUCTS

PRODUCT	PACK SIZE	CAT NO.
Agarose	100 g	BIO-41026
MyTaq™ HS DNA Polymerase	250 Units	BIO-21111
HyperLadder™ 1 kb	500 Lanes	BIO-33026
HyperLadder™ 100 bp	500 Lanes	BIO-33030

D. PRODUCT WARRANTY AND DISCLAIMER

Meridian warrants that its products will conform to the standards stated in its product specification sheets in effect at the time of shipment. Meridian will replace free of charge any product that does not conform to the specifications. This warranty limits Meridian's liability only to the replacement of the product.

NOTES

Technical Support
For technical assistance or more information on these products, please contact us at mbi.tech@meridianlifescience.com or call us on +49 (0) 3371 60222 03

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