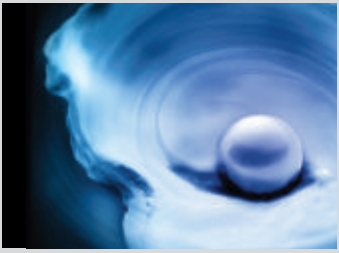

ISOLATE II
HT 96 Clean-Up Kit

Product Manual

BIOLINE

A Meridian Life Science® Company

ISOLATE II HT 96 Clean-Up Kit

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2. DESCRIPTION

2.1 INTRODUCTION

The ISOLATE II HT 96 Clean-Up Kit is specially designed for the rapid high-throughput purification of PCR fragments using either manual or automated protocols. The PCR samples are applied to an ultrafiltration membrane and a vacuum or centrifugal force is applied. The filtration process rapidly and efficiently removes small molecule contaminants such as salts, primers (<20mers) and unincorporated nucleotides, whilst retaining PCR products on the membrane. The ISOLATE II HT procedure does not require use of chaotropic salts for nucleic acid binding or ethanol-based wash steps.

Purified PCR products are recovered from the membrane surface by adding a low salt buffer or nuclease-free water. It is possible to process PCR sample volumes from 20-300µl using either a manual or automated protocol. PCR products are typically purified with a recovery rate of up to 95%. The purified nucleic acids can subsequently be used directly for a range of downstream applications such as DNA sequencing, microarray spotting, labeling, cloning, or restriction digestion.

Please read this manual carefully to familiarize yourself with the ISOLATE II HT 96 Clean-Up system before starting (also available on www.bioline.com). More experienced users can refer to the bench-top protocol for quick referencing during the protocol.

2.2 CLEAN-UP TECHNOLOGY

ISOLATE II HT 96 Clean-Up Basic Principle

Transfer PCR samples to wells of ISOLATE II HT 96 plate

Ultrafiltration by vacuum/centrifuge

Wash membrane with nuclease-free water*

**Optional step for vacuum-based processing*

Recover purified PCR samples

Add recovery buffer or nuclease-free water

2.3 RECOVERY OF PURIFIED PCR PRODUCTS

Purified PCR products can be recovered directly from the membrane using a recovery buffer (5mM Tris/HCl) or nuclease-free water.

For the manual procedure, the recovery volume should be at least 25µl. Use a multichannel pipette to recover the buffer or water containing the purified PCR products completely from the wells. The tips may touch the membrane slightly during the manual recovery process.

For the automated procedure, a minimum recovery volume of 50µl is recommended to improve both recovery and well-to-well consistency (see section 2.5). It is important to collect the recovery buffer/water completely from the membrane for optimal recovery of PCR products.

With the ISOLATE II HT 96 Clean-Up membrane it is possible to touch the membrane with the tips during the recovery process without the risk of damage. The sturdy ultrafiltration membrane facilitates easy recovery of purified PCR products. Damage would result in the risk of co-recovering small membrane parts, a common problem with other ultrafiltration membranes. These parts might interfere with subsequent applications, especially capillary sequencing and microarray spotting.

Enhanced recovery of DNA can be facilitated either by a short incubation at room temperature, mixing, or by using a plate shaker after the addition of recovery buffer or nuclease-free water; this is especially recommended for PCR products ≥500bp.

2.4 COMPATIBLE VACUUM MANIFOLDS

The ISOLATE II HT 96 Clean-Up plate is designed to be compatible with the following vacuum manifolds:
QIAvac 96 (Qiagen), Vac-Man® 96 (Promega), Aurum™ (Bio-Rad), NucleoVac 96 (Macherey-Nagel) and MultiScreen® (Millipore). The Perfect VAC (Eppendorf) manifold is not compatible.

2.5 AUTOMATION OF ISOLATE II HT 96 CLEAN-UP KIT

The ISOLATE II HT 96 Clean-Up Kit is compatible with common automated liquid handling instruments.

For automated systems, it is recommended to use a recovery volume of ≥50µl, to improve both DNA recovery and well-to-well consistency. Smaller volumes are possible, but may lead to poor perfomance. Recovery can be improved either by mixing, incubation, or the use of a plate shaker (see section 7.1).

A critical step in the protocol is the effective recovery of PCR products from the membrane. Needles/tips must be as close to the membrane as possible during the recovery step to recover the buffer or nuclease-free water completely. Slight touching of the membrane will not damage the membrane, but may block the needles/disposable tips during the recovery process, resulting in a reduced recovery. The height of the needles/disposable tips above the membrane should be carefully calibrated for each individual platform for best results.

Ensure that the vacuum is released before recovering the PCR products and adjusting the height of the needles/disposable tips, since the ISOLATE II HT 96 Clean-Up Plate has a lower position inside the manifold under vacuum. This may result in a loss of about 20–30% of PCR products.

Please contact your instrument supplier regarding hardware, software, setup instructions and selection of available protocols.

3. STORAGE

The ISOLATE II HT 96 Clean-Up Kit should be stored dry and at room temperature (18–25°C). The kit can be stored for up to one year from purchase without any decrease in performance or separation quality.

4. SAFETY INFORMATION

All kit components are non-hazardous.

5. PRODUCT SPECIFICATIONS

The ISOLATE II HT 96 Clean-Up Kit is designed for both manual and automated use. It is suitable for the rapid manual clean-up of PCR fragments using vacuum manifolds (section 7.1), or microplate centrifuges (see section 7.2). The kit can also easily be adapted for automated liquid handling instruments (see section 2.5).

ISOLATE II HT96 CLEAN-UP PLATE SPECIFICATIONS	
Typical DNA recovery	75–95%
Fragment size	>150bp
PCR reaction mix per well	20–300µl*
Purity of cleaned PCR products (A_{260}/A_{280})	≥1.7–1.8
Primers (<20mer)	Removed
Time required (96 samples)	~20 min (manual), ~15 min (automated)
Recovery volume	≥25µl (manual), ≥50µl (automated)

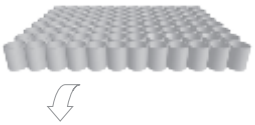
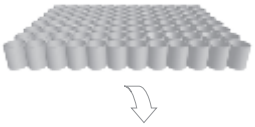
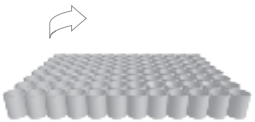
**If processing up to 300µl, the sample has to be loaded stepwise and filtration times will need to be increased.*

6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY THE USER

- Recovery buffer (5mM Tris/HCl, pH 8.5) or nuclease-free water (recommended)
- Multichannel pipette and tips
- Self-adhesive 96-well plate seals
- Compatible vacuum manifold, or microtitre plate centrifuge (capable of accelerations of at least 4,500 x g) or automated liquid handling instrument
- Microplate shaker (optional)
- 96-well square or round well block for waste collection during centrifuge processing

7. GENERAL PURIFICATION PROTOCOL

Clean-up Procedure

Transfer PCR samples to ISOLATE II HT 96 Clean-Up Plate	20-300µl	
Filter contaminants by vacuum or centrifuge	-0.4 to -0.6 bar* 10–15 min or 4,500 x g, 10–15 min	
Wash membrane**	100µl water (nuclease-free) -0.4 to -0.6 bar* 10–15 min or 4,500 x g, 5–10 min	
Recover purified PCR samples	25–100µl recovery buffer or water (nuclease-free)	

** Reduction of atmospheric pressure*
*** Optional for vacuum processing; essential for centrifuge processing*

7.1 STANDARD PROTOCOL USING A VACUUM

Important notes

This protocol is for the purification of up to 96 PCR samples (PCR reaction volumes of 20–100µl), using a manual procedure or an automated instrument. For PCR reaction volumes of up to 300µl filtration times have to be increased.

- Use of the ISOLATE II HT 96 Clean-Up Plates requires a compatible vacuum manifold (see 2.4).
- For automation, contact your supplier regarding hardware, software, setup instructions, and selection of available protocols.
- Overall filtration time depends on sample volume and vacuum strength. Apply a vacuum of up to -0.6 bar.
- DNA recovery can be performed with a microplate shaker, and is particularly recommended for PCR products ≥500bp. When using a shaker, the dispensed recovery volume should be ≥50µl.

- Alternatively, a multichannel pipette can be used to dissolve samples by pipetting up and down for at least 10 times. The recovery volume should be at least 25µl.
- Recovery buffer or nuclease-free water must be supplied by the user.

Calibrating a microplate shaker for DNA recovery

Calibrate the microplate shaker to prevent well to well cross-contamination by following the steps below:

A Apply 50–100µl of colored dyed water (e.g. bromophenol blue) to several wells.

B Position the 96-well plate on the microplate shaker.

C Set shaker at low speed setting for 30s. Turn off shaker and check plate surface for liquid.

D Slowly increase speed, shake for an additional 30s and check plate surface for liquid again.

E The recommended shaking speed for recovery is the maximum speed where no liquid splashing is observed.

Clean-up procedure

1 **Place ISOLATE II HT 96 Clean-Up Plate on vacuum manifold**

2 **Transfer PCR samples to ISOLATE II HT 96 Clean-Up Plate**

Note: Smaller PCR reaction volumes should be made up to 100µl with nuclease-free water to facilitate uniform plate loading. Carefully dispense samples onto the membrane, taking care not to pipette any liquid onto the inner well walls. Any unused plate wells may be left unsealed.

3 **Apply vacuum to remove contaminants by ultrafiltration**

Note: Apply a vacuum of up to -0.6 bar. Typically, a 100µl sample volume requires 10-15 min vacuum time. Processing PCR sample volumes up to 300µl requires increased filtration times. When all the solution has passed the membrane, apply vacuum for a further 30-60s to allow excess liquid to drain.

4 **Optional wash step**

Release the vacuum for 1-1.5 min. Add 100µl nuclease-free water into each well and apply the vacuum (-0.4 to -0.6 bar) until the water passes the membrane. Apply vacuum for a further 30-60s.

Note: DNA purity after recovery is normally sufficient for most applications without this wash step being needed. This step should be performed if a higher purity is required for a specific application.

5 **Add recovery buffer or nuclease-free water to plate wells**

Ensure vacuum has been turned off (1-1.5 min) before adding recovery buffer/water. Add 25–100µl (depending on DNA concentration required) of recovery buffer or nuclease-free water directly onto the membrane of the 96-well plate. Incubate for at least 5 min at room temperature.

Note: Ensure vacuum is switched off when dispensing the recovery buffer or water. The dispensed volume should be ≥50µl if using a shaker in step 6a, or ≥25µl if using a multichannel pipette in 6b.

6 **Dissolve purified DNA according to step 6a or 6b**

6a Shake ISOLATE II HT 96 Clean-Up Plate on a microplate shaker for 2–5 min at calibrated speed (see Calibrating a microplate shaker for DNA recovery).
Note: Ensure ISOLATE II HT 96 Clean-Up Plate is firmly secured on top of the shaker.

6b Alternatively, pipette samples up and down at least 10 times using a multichannel pipette.

7 **Recover purified PCR products by pipetting the sample out of each well**

7.2 STANDARD PROTOCOL USING A CENTRIFUGE

Important notes

This protocol is for manual processing using a microplate centrifuge and is designed for the purification of up to 96 PCR samples (PCR reaction volumes of 20–100µl). For PCR reaction volumes of up to 300µl, centrifugation times have to be increased.

- A suitable microplate centrifuge is required. The centrifuge buckets must be able to hold the ISOLATE II HT 96 Clean-Up Plate on top of a suitable plate for waste collection (e.g. square-well block, round-well block, not provided).
- Standard microtitre plates must not be used for waste collection as they break under the g-forces required to process the ISOLATE II HT 96 Clean-Up Plate.
- Ensure buckets can hold the ISOLATE II HT 96 Clean-Up Plate and waste collection plate. This can be tested before the experiment begins by placing a standard microtitre plate on top of the appropriate waste collection plate and see if this fits into the bucket. If using a standard square-well block for waste collection, the sandwich height is 58mm.
- For processing of the ISOLATE II HT 96 Clean-Up Plates, a centrifugal force of 4,500 x g is recommended. Lower g-forces will increase filtration times significantly.
- The washing step is essential if ISOLATE II HT 96 Clean-Up is used for the centrifugation protocol.

Clean-up procedure

1 **Transfer the PCR samples (20–100µl) to the ISOLATE II HT 96 Clean-Up Plate**
Unused wells of the ISOLATE II HT 96 Clean-Up Plate may be left open; sealing is not required.

2 **Remove contaminants by ultrafiltration**
Place the ISOLATE II HT 96 Clean-Up Plate onto a suitable waste collection plate (e.g. square-well block).
Place the sandwich in the centrifuge and spin at 4,500 x g.
Note: Typically, centrifugation for 5–10 min for a sample volume of 50–100µl is sufficient.

3 **Wash step (essential)**

Add 100µl nuclease-free water into each well of the ISOLATE II HT 96 Clean-Up Plate. Place on top of the waste collection plate and centrifuge for 5–10 min at 4,500 x g.

Note: When the ISOLATE II HT 96 Clean-Up Plate is used under centrifugation, the washing step is essential. Approximately 3–5µl of PCR sample (containing salts, primers, dNTPs) will remain on top of the membrane after the initial centrifugation step. To avoid contamination of the purified PCR sample, the washing step is essential to remove the contaminants.

4 **Add recovery buffer or nuclease-free water to plate wells**

Dispense an appropriate volume (25–100µl) of recovery buffer or nuclease-free water directly onto the membrane of the ISOLATE II HT 96 Clean-Up Plate. Incubate for at least 5 min at room temperature.

5 **Dissolve purified DNA according to step 5a or 5b**

5a Shake ISOLATE II HT 96 Clean-Up Plate on a microplate shaker for 2–5 min at calibrated speed (see section 7.1).

Note: Ensure ISOLATE II HT 96 Clean-Up Plate is firmly secured on top of the shaker.

5b Alternatively, pipette samples up and down at least 10 times.

6 **Recover purified PCR products by pipetting the sample out of each well**

8. TROUBLESHOOTING GUIDE

LOW DNA RECOVERY	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Volume of recovery buffer or nuclease-free water not sufficient	Increase amount of recovery buffer/nuclease-free water to at least 25µl for manual use and at least 50µl for automated use.
Insufficient mixing or shaking during recovery step	Increase number of mixing steps.
	Increase incubation time.
	Check microplate shaker speed settings are optimal.
DNA fragments dehydrated onto membrane	Incubate recovery buffer/ nuclease-free water on membrane for 15–30 min at room temperature to allow DNA to rehydrate before recovery.
PCR fragment <150bp	Use ISOLATE II PCR and Gel kit for purifiying small PCR products.
SAMPLE CONTAMINATION	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Samples not filtered completely	Allow the samples to pass membrane completely. Wait until the membrane looks totally dry. Ensure the vacuum is applied at sufficient pressure and time. Ensure centrifugation is carried out at 4500 x g for sufficient time.
Residual PCR samples left on the side of the wells	Carefully transfer PCR samples directly onto the membrane. Ensure samples do not stick to the side of the well, as contaminants may become co-recovered. Avoid tips touching well sides during automated steps; ensure instrument is calibrated correctly. Perform optional washing step.
PCR contains detergents	Certain detergents e.g. Triton™ X-100 may not be totally removed by ultrafiltration. Avoid detergent in PCR reaction set-up where possible.
No wash step performed under centrifuge-based processing	Perform wash step to remove contaminants e.g. salts, primers, dNTPs.

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 HT 96 Clean Up-Kit	10 Plates	BIO-52061

C. ASSOCIATED PRODUCTS

PRODUCT	PACK SIZE	CAT NO.
PCR Water (nuclease free)	10 x 10ml	BIO-37080
Seal plate (adhesive)	100 Seals	PCR-SP-S
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

D. PRODUCT WARRANTY AND DISCLAIMER

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

A Meridian Life Science® Company

www.bioline.com

United Kingdom

Bioline Reagents Ltd
16 The Edge Business Centre
Humber Road
London NW2 6EW
Tel: +44 (0)20 8830 5300
Fax: +44 (0)20 8452 2822
email: info.uk@bioline.com

Germany

Bioline GmbH
Im Biotechnologiepark, TGZ 2
D-14943 Luckenwalde
Tel: +49 (0)3371 68 12 29
Fax: +49 (0)3371 68 12 44
email: info.de@bioline.com

Australia

Bioline (Aust) Pty Ltd
PO Box 122
Alexandria NSW 1435
Tel: +61 (0)2 9209 4180
Fax: +61 (0)2 9209 4763
email: info.aust@bioline.com

USA

Bioline USA Inc.
305 Constitution Drive
Taunton MA 02780
Tel: +1 508 880 8990
Fax: +1 508 880 8993
Order Toll Free: +1 888 257 5155
email: info.us@bioline.com