May 2017

UltraClean® 96 PCR Cleanup Kit Handbook

For high-throughput DNA cleanup from PCR, sequencing and other enzyme reactions

Sample to Insight

QIAGEN

Contents

Kit Contents

UltraClean 96 PCR Cleanup Kit	**(384)**
Catalog no.	**12596-4**
Number of preps	**384**
Solution SB	250 ml
SpinClean	4 x 30 ml
Solution EB	51 ml
QIAamp 96 Plate	4
S-Blocks	2
AirPore Tape Sheets	25
Sealing Tapes	16
Elution Microtubes, CL	4
Caps for Elution Microtubes	50 x 8
Quick Start Protocol	1

Storage

The UltraClean 96 PCR Cleanup Kit reagents and components should be stored at room temperature (15–25°C).

Intended Use

All UltraClean products are intended for research applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

WARNING: SpinClean® and Solution SB contain alcohol and are flammable.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

The sample preparation waste contains guanidine hydrochloride from Buffer SB (guanidine hydrochloride) which can form highly reactive compounds when combined with bleach.

If liquid containing this buffer is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water and then with 1% (v/v) sodium hypochlorite.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of UltraClean 96 PCR Cleanup Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The UltraClean 96 PCR Cleanup Kit is designed to purify PCR products directly from a PCR or enzyme reaction in three minutes without running a agarose gel. This kit is compatible with downstream sequencing of PCR reactions or applications where efficient removal of PCR primers is critical. All reagents are optimized to remove primers.

Principle and Procedure

Plasmid DNA is isolated in 96-well plates. Adding the binding buffer, the silica membrane spin filter selectively binds the PCR or reaction product. Unwanted reaction components pass through the filter plate during centrifugation. Desired product is then washed and recovered from the spin filter plate in a DNA-free Tris buffer. The resulting DNA can be used for any downstream application.

Recovered DNA is in the range of 60 bp to 10 kb. Each QIAamp® 96 Plate can bind 20 µg of DNA for a final DNA volume of 100 µl. Recovery rates range from 80 to 100% of material for downstream applications.

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, consult the appropriate safety data sheets (SDSs) available from the product supplier.

- Centrifuge (4,500 x *g*) capable of spinning two stacked 96 well blocks (13 cm x 8.5 cm x 7.5 cm)

  Note: If the centrifuge used has a maximum speed under 4500 x *g*, see the Troubleshooting Guide.
- Multi-channel pipettor (volume 10 µl – 1000 µl)

Protocol

Notes before starting

- Check to ensure centrifuge is capable of spinning two stacked 96-well blocks (13 cm x 8.5 cm x 7.5 cm)
- **Note:** if the centrifuge to be used has a maximum speed under 4500 x g, see the Troubleshooting Guide.
- Shake to mix Solution SB before use
- Ensure that the centrifuge being used will accommodate the plates in this kit. Stack a QIAamp 96 Plate on top of an S-Block and place them in the plate holder rotor. **DO NOT start the centrifuge**. Turn the centrifuge slowly by hand to make sure that the stacked plates fit inside the centrifuge.
- Make sure you have a multi-channel pipettor that can accommodate 10 µl–1000 µl.
- This protocol assumes you will be processing 192 samples (two 96-well preps). If you plan to process fewer than 192 samples, divide your samples between the two plates evenly to ensure the centrifuge is balanced (see the Troubleshooting Guide).

Procedure

1. Shake to mix Solution SB before use. Add 5 volumes of Solution SB to PCR reaction.
2. Mix well by pipetting.
   Note: If an oil overlay was used, there will be two layers; the top layer is oil.
3. Place an S-Block under a QIAamp 96 Plate. The S-Block is reusable.
4. Transfer PCR/Solution SB mixture to the QIAamp 96 Plate. Avoid transferring any oil.
   Note: Any unused wells in the QIAamp 96 Plate may be used later.
5. Seal the wells with a piece of AirPore Tape Sheet.
6. Centrifuge the QIAamp 96 Plate/S-Block at 4500 x *g* for 3 min.
7. Remove the QIAamp 96 Plate and discard the flow-through from the S-Block.
8. Remove AirPore Tape Sheet. Place the QIAamp 96 Plate on the same S-Block.

9. Add 300 µl SpinClean to the QIAamp 96 Plate and seal the plate with a new piece of AirPore Tape Sheet.
10. Centrifuge at 4500 x *g* for 3 min.
11. Remove the QIAamp 96 Plate and discard the flow-through from the S-Block.
12. Replace the QIAamp 96 Plate on the same S-Block.
13. Centrifuge again at 4500 x *g* for 6 min.
14. Carefully transfer the QIAamp 96 Plate to a new rack of Elution Microtubes (provided).
15. Remove the AirPore Tape Sheet and allow to air dry for 10 min at room temperature.
16. Add 100 µl of Solution EB or sterile water to the center of the white spin filter membranes of the QIAamp 96 Plate.
    Note: Using Solution EB or water will not affect DNA yield. DNA is more stable when stored in Solution EB.
17. Seal the QIAamp 96 plate with a new AirPore Tape Sheet and centrifuge at 4500 x *g* for 3 min.
18. Remove the QIAamp 96 Plate from the Elution Microtubes. Seal the Elution Microtubes with provided caps. The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–20°C to –80°C) as Solution EB does not contain EDTA.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies. For contact information, visit **www.qiagen.com**.

		Comments and suggestions
Samples		
a)	If fewer than 96 samples are being processed, full QIAamp 96 Plate will not be used	1) Seal entire QIAamp 96 Plate with Sealing Tape 2) Using a scalpel blade, cut out only the wells containing the PCR/Solution SB mixture 3) Keep Sealing Tape on unused wells throughout the rest of the protocol
b)	Resultant DNA is too dilute	The final volume will be 100 µl. If this is too dilute for your purposes, add 4 µl of 5M NaCl and mix. Then add 200 µl of 100% cold ethanol. Mix. Centrifuge at 10,000 x *g* for 5 minutes. Decant all liquid. Dry residual ethanol in a speed vac or desiccator or ambient air. Resuspend precipitated DNA in desired volume.
c)	DNA floats out of the well when loaded on a gel	Residual SpinClean is in the final sample. Prevent this by being careful in step 14 of centrifuge protocol. Use ethanol to precipitate and remove residues of SpinClean. (See procedure for Concentrating DNA above)
d)	Low recoveries noted	Low recoveries can be due to not mixing Solution SB well with your sample in step 2. Incomplete removal of SpinClean can also reduce yields. Make sure your centrifuge is spinning at a minimum of 2500 x *g*.
e)	EDTA elution buffer inhibits subsequent enzymatic reactions	Repurify the sample with this kit and use Solution EB provided for the elution step.
Centrifuge		

		Comments and suggestions
a)	Centrifuge has a maximum speed less than 4500 x *g*	Multiply the protocol time and speed to determine total x *g*. Divide the total by the maximum speed of your centrifuge (round up if necessary). This will be the number of minutes your centrifuge will need to run to achieve the appropriate overall force. Example: 10 minutes at 4500 x *g* = 45,000. If your centrifuge has a maximum speed of 2500 x *g*, divide 45,000 ÷ 2500 = 18 minutes of centrifugation.

Ordering Information

Product	Contents	Cat. no.
UltraClean 96 PCR Cleanup Kit	For 4 preps: For high-throughput DNA cleanup from PCR, sequencing and other enzyme reactions	12596–4
Related Products	**Contents**	**Cat. no.**
QIAquick PCR Purification Kit (50)	For purification of 50 PCR reactions: For purification of up to 10 µg PCR products, 100 bp to 10 kb	28104
QIAquick PCR Purification Kit (250)	For purification for 250 PCR reactions: For purification of up to 10 µg PCR products, 100 bp to 10 kb	28106
QIAquick Gel Extraction Kit (50)	For gel extraction or cleanup of 50 reactions: For gel extraction/cleanup of up to 10 µg DNA (70 bp to 10 kb) from enzymatic reactions	28704
QIAquick Gel Extraction Kit (250)	For gel extraction or cleanup of 250 reactions: For gel extraction/cleanup of up to 10 µg DNA (70 bp to 10 kb) from enzymatic reactions	28706

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Notes

Limited License Agreement for UltraClean 96 PCR Cleanup Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see **www.qiagen.com**.

Ordering **www.qiagen.com/shop** | Technical Support **support.qiagen.com** | Website **www.qiagen.com**

1104554 HB-2251-001 04/2016