April 2018

MagAttract® PowerClean® DNA Kit Handbook

For automated removal of PCR inhibitors from previously purified DNA using magnetic bead technology

Sample to Insight

QIAGEN

Contents

Kit Contents

MagAttract PowerClean DNA Kit	**(384)**
Catalog no.	**27900-4-KF**
Number of preps	**4 x 96**
Solution CU	25 ml
Solution IR	2 x 14 ml
ClearMag® Binding Solution	200 ml
ClearMag Zorb Reagent	9 ml
Solution EB	51 ml
96 Well Plate (Sterile)	4
Sealing Tape	16
Quick Start Protocol	1

Storage

All reagents and components of the MagAttract PowerClean DNA Kit can be stored at room temperature (15–25°C) until the expiration date printed on the box label.

Intended Use

All MagAttract products are intended for molecular biology 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.

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

Solution CU contains guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing this buffer is spilt, clean with a 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 MagAttract PowerClean DNA Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The MagAttract PowerClean DNA Kit is optimized for use with the Thermo Scientific™ KingFisher™ Flex and KingFisher Duo platforms.

The kit uses Inhibitor Removal Technology® (IRT) and ClearMag magnetic beads to provide researchers with a high-throughput, automated method for removing PCR-inhibiting contaminants from up to 20 µg of previously isolated genomic DNA. Using the same DNA purification chemistry as our popular PowerClean® Pro DNA Cleanup Kit, the MagAttract PowerClean DNA Kit streamlines DNA purification by enabling truly hands-off, walk-away workflows.

Principle and procedure

Proprietary DNA Cleanup reagents are used to purify archived or previously isolated DNA samples. Inhibitors are selectively removed from DNA solutions via flocculation and the resulting DNA-containing supernatant is combined with ClearMag Beads and a proprietary binding buffer in KingFisher deep-well plates. DNA is captured on ClearMag Beads, washed with ethanol and eluted. Purified DNA is ready for downstream applications, including PCR.

The MagAttract PowerClean DNA Kit can be used to remove contaminants that discolor samples and prevent accurate DNA quantification. The kit can also be used to remove PCR-inhibiting substances, such as humic acids, heme, polysaccharides, polyphenols, fulvic acids, dyes and certain inhibitory ions. The resulting high-purity DNA ensures successful downstream applications, including restriction digestion, PCR amplification and next-generation sequencing (NGS).

The MagAttract PowerClean DNA Kit has been validated with DNA isolated from a variety of problematic sources including soil, water, plants, stool and biofilms.

The MagAttract PowerClean DNA Kit is optimized for use with the Thermo Scientific KingFisher Flex and KingFisher Duo platforms. Other open platform robots may be used with this kit. However, you may need to contact your local field application scientist or the manufacturer of your robot for help in adapting this protocol to that system.

The order of placement of components and reagents on the robotic deck are described in the downloaded software.

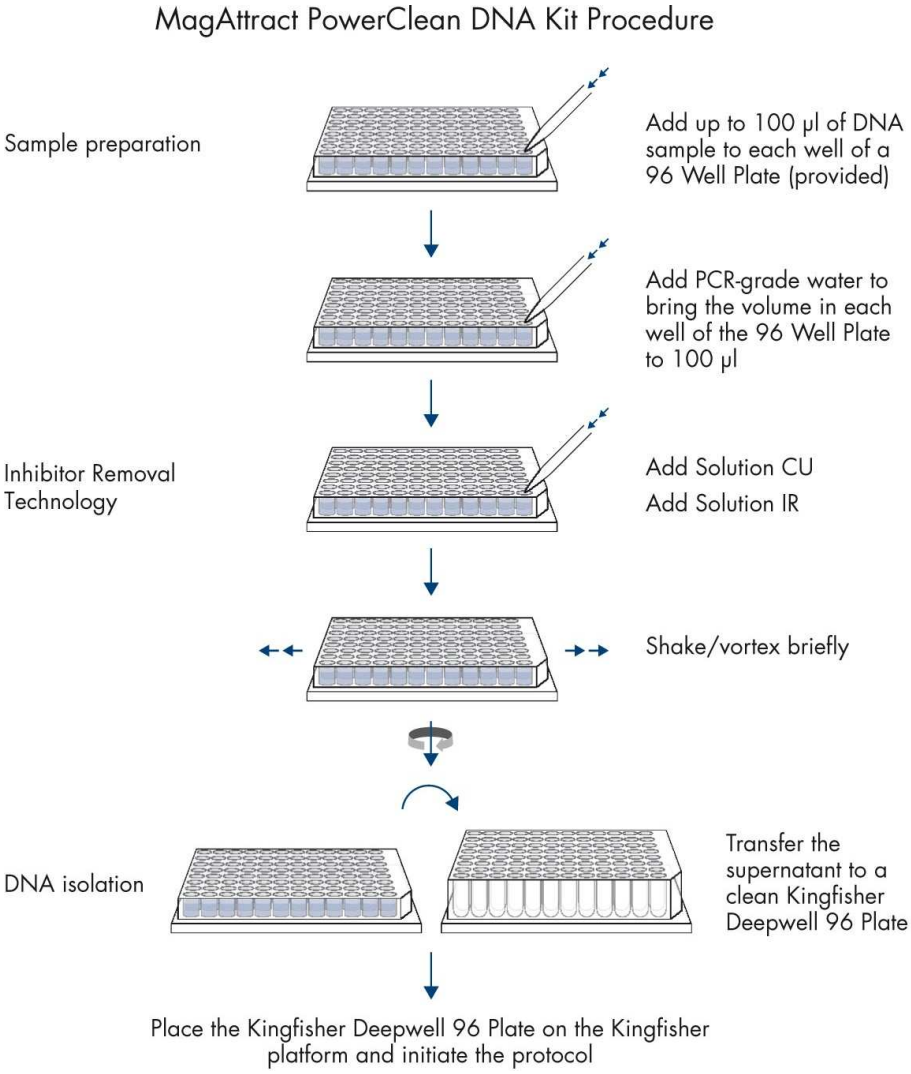

Figure 1. MagAttract PowerClean DNA Kit procedure.

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 capable of handling two 96 well blocks (13 cm x 8.5 cm x 1.4 cm) at 4500 x *g*
  Note: If your centrifuge has a maximum speed less than 4500 x *g*, please refer to the Troubleshooting Guide.
- Multi-channel pipettors (50–1000 µl)
  Note: A 12 channel pipettor is required if multichannel pipetting is desired when using the KingFisher Duo platform.
- Single-channel pipettors (10–1000 µl)
- Orbital shaker for 96 well plates capable of 450 rpm
- Vortex-Genie® 2 Vortex
- Please contact your Thermo Fisher Scientific representative for KingFisher plastic disposables specific to the platform being used
- Reagent reservoirs (5–300 ml)
- 15 ml conical tubes (for Kingfisher Duo protocol)
- 50 ml conical tubes (for Kingfisher Flex protocol)
- 100% ethanol
- PCR-grade water

Protocol

Important points before starting

- This kit was designed to work with up to 20 µg of input DNA and has been used successfully with as little as 20 ng. Using more than 20 µg will decrease the efficiency of inhibitor removal and can lead to loss of nucleic acid recovery.
- For best results, input DNA samples should be in water or Tris buffer, pH 8.0. We do not recommend using this kit with buffers containing >1 mM EDTA.
- The Kingfisher Flex protocol requires 300 ml of 100% ethanol for each full 96 Well Plate being processed. The KingFisher Duo requires 40 ml of 100% ethanol for each 12 wells being processed.

Procedure

1. Add up to 100 µl of DNA sample to each well of a 96 Well Plate (provided). If less than 100 µl is added, adjust the sample volume to 100 µl using PCR-grade water (user provided).
2. Add 50 µl of Solution CU to each well containing DNA. Mix by repeated pipetting (3X). **Note:** If processing a full 96 Well Plate, dispensing Solution CU and Solution IR (step 3) using a multichannel pipettor or a repeating pipettor may reduce processing time.
3. Add 50 µl of Solution IR to each well containing DNA. Seal the plate with Sealing Tape and mix the contents in the wells by gently tapping on the sides of the sealed plate.
4. Centrifuge the plate at 4500 x *g* for 6 min at room temperature with an appropriately weighed balance plate.
5. To run the DNA purification protocol on the KingFisher Flex, go to page 11. If using the KingFisher Duo, go to page 13.

KingFisher Flex

Continued from step 5 on page 10

6. Resuspend ClearMag Beads (Zorb Reagent) by vortexing the bottle. For each 96 Well Plate to be processed, combine 45 ml of ClearMag Binding Solution and 2 ml of ClearMag Beads in a 50 ml conical tube (user provided). Mix well to obtain a homogeneous dispersion of beads.
7. Add 470 µl of the ClearMag Beads/Binding Solution to each well of a KingFisher Flex Microtiter Deepwell 96 plate.
   Note: Work quickly; the ClearMag Beads will slowly settle over time. Maintain the beads in suspension for uniform distribution to each well
8. Remove and discard the Sealing Tape from the plate centrifuged in step 4. Avoiding the pellet, transfer the entire volume (expect 150–190 µl) of supernatant from each well to the respective well in the KingFisher Flex Microtiter Deepwell 96 plate from step 7.
   Note: We recommend using an 8 tip or 12 tip multichannel pipettor to reduce total processing time. Placing the 96 Well Plate on top of a colored (i.e., non-white) support may aid in visualizing the pellet at the bottom of each well.
   Note: To avoid disrupting the pellet while transferring the supernatant, we recommend that you insert pipette tips along the wall of each well during the aspiration step. By placing pipette tips in physical contact with the well wall and sliding the tips to the bottom of the wall, the tips will avoid contact with the pellet. Aspirating fluid from the center of the wells is not recommended.
9. Open the KingFisher Flex-specific PowerMag® DNA Cleanup program on your instrument.
10. Place the KingFisher Flex Microtiter Deepwell 96 plate containing the DNA samples and ClearMag Beads/Binding Solution (from step 8) onto the robotic deck at the specified location indicated in the PowerMag DNA Cleanup program.

11. Place 1 ml of 100% ethanol (user provided) into each well of three clean KingFisher Flex Microtiter Deepwell 96 plates. Place the plates on the deck at the specified locations indicated in the PowerMag DNA Cleanup program.
12. Place 50–100 µl of Solution EB into each well of a KingFisher 96 KF Elution plate and place on the deck at the specified location. Initiate the KingFisher PowerMag DNA Cleanup robotic program.
13. When the robotic program is complete, cover the wells of the KingFisher 96 KF Elution plate with an appropriate storage seal (user provided). The DNA is now ready for downstream applications.

    Note: We recommend storing DNA frozen (–15 to –30°C or –65 to –90°C) as Solution EB does not contain EDTA.

KingFisher Duo

Continued from step 11 on page 12

6. Resuspend ClearMag Beads (Zorb Reagent) by vortexing the bottle. For each set of 12 samples to be processed, combine 5.625 ml of ClearMag Binding Solution and 250 µl of ClearMag Beads in a 15 ml conical tube (user provided). Mix well to obtain a homogeneous dispersion of beads.
   Note: Work quickly; the ClearMag Beads will slowly settle over time. Maintain the beads in suspension for uniform distribution to each well.
7. Add 470 µl of the ClearMag Beads/Binding Solution to each well in Row A of a KingFisher Flex Microtiter Deepwell 96 plate.
8. Remove and discard the Sealing Tape from the plate centrifuged in step 4. Avoiding the pellet, transfer the entire volume (expect 150–190 µl) of supernatant from each well to the respective well in the KingFisher Flex Microtiter Deepwell 96 plate from step 7.
   Note: We recommend using an 8 tip or 12 tip multichannel pipettor to reduce total processing time. Placing the 96 Well Plate on top of a colored (i.e., non-white) support may aid in visualizing the pellet at the bottom of each well.
   Note: To avoid disrupting the pellet while transferring the supernatant, we recommend that you insert pipette tips along the wall of each well during the aspiration step. By placing pipette tips in physical contact with the well wall and sliding the tips to the bottom of the wall, the tips will avoid contact with the pellet. Aspirating fluid from the center of the wells is not recommended.
9. Place a KingFisher Duo 12-tip comb (user provided) into the second row (B) of the KingFisher Flex Microtiter Deepwell 96 plate.
10. Add 1 ml of 100% ethanol (user provided) to each well of the next three rows (C, D and E) of the KingFisher Flex Microtiter Deepwell 96 plate and place on the deck.
11. Add 50–100 µl of Solution EB to each well of a KingFisher Duo Elution Strip (user provided) and place the strip on the deck.
12. Initiate the KingFisher PowerMag DNA Cleanup robotic program.

13. Upon completion, cover the wells of the KingFisher Duo Elution Strip with an appropriate storage seal (user provided). The DNA is now ready for downstream applications.

    Note: We recommend storing DNA frozen (–15 to –30°C or –65 to –90°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
Sample processing		
a)	Amount of sample to process	This kit is designed to process up to 100 µl of DNA (20 µg maximum). For inquiries regarding the use of larger sample amounts, please contact QIAGEN Technical Services (**support.qiagen.com**) for suggestions.
b)	Using a centrifuge with 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 by 2500 = 18 minutes of centrifugation.
DNA		
a)	DNA does not amplify	Check DNA integrity using gel electrophoresis and DNA concentration using appropriate methods (see "Quantifying DNA" section on page 16). Typically, 10–100 ng of DNA template is added per reaction, although more or less may be needed depending on the reaction conditions, enzyme activity and copy number of the target sequence. If DNA does not amplify after altering the amount of template per reaction, please call QIAGEN Technical Services (**support.qiagen.com**) for suggestions.
b)	Eluted DNA is brown	We have not observed any coloration in the DNA isolated using the MagAttract PowerClean DNA Kit. If you observe colorations in eluted DNA, please contact QIAGEN Technical Services (**support.qiagen.com**).

		Comments and suggestions
c)	Quantifying DNA	UV-VIS spectrophotometric analysis of nucleic acids (e.g., using NanoDrop®) reports the total absorbance profile of a sample (i.e., the contributions of DNA/RNA and any non-nucleic acid, UV-absorbing compounds present in the sample), which makes UV-VIS spectrophotometry susceptible to reporting DNA concentrations higher than the actual value. Therefore, we recommend using UV-VIS spectrophotometry to assess sample quality (260/280, 260/230 ratios) and using a nucleic acid specific assay (e.g., Qubit™) for accurate DNA quantification. **Note:** We have determined that a small amount of ClearMag Binding Solution will be present in the eluted DNA. This residual Binding Buffer will not negatively impact PCR, NGS library preparation or any other downstream process but will inflate DNA concentration measurements made using UV-VIS spectrophotometry. If only UV-Vis spectrophotometry is used to quantify isolated DNA, we recommend running a 'blank' DNA isolation alongside the samples when using this kit. An appropriate blank is 450 µl of ClearMag Binding Solution, 20 µl ClearMag Beads (Zorb Reagent) and 200 µl PCR-grade water. Run this blank alongside the other samples and use the same ethanol wash and elution conditions. During DNA quantification, subtract the ng/µl value obtained for the blank (typically 10 ng/µl) from the other measured samples to obtain more accurate values.
d)	Concentrating eluted DNA	DNA may be concentrated by adding 5 µl of 3 M NaCl and inverting 3–5 times to mix. Next, add 2.5 volumes of 100% ice-cold ethanol and invert 3–5 times to mix. Incubate at –15 to –30°C for at least 10 minutes to overnight. Centrifuge at 13,000 x *g* for 15 minutes. Decant all liquid. Wash the nucleic acid pellet with 70% cold ethanol and centrifuge at 13,000 x *g* for 10 minutes to re-pellet. Decant all liquid. Briefly dry residual ethanol in a speed vac or ambient air. Avoid over-drying the pellet or resuspension may be difficult. Resuspend precipitated nucleic acids in desired volume of PCR-Grade Water (cat. no. 17000-10) or 10 mM Tris. **Note:** This procedure must be done after transferring the eluted samples to individual microcentrifuge tubes.
e)	Storing DNA	DNA is eluted in Solution EB (10 mM Tris) and must be stored at –15 to –30°C to prevent degradation. DNA can be eluted in TE without loss, but the EDTA may inhibit downstream reactions such as PCR and automated sequencing. DNA may also be eluted in sterile PCR-Grade Water (cat. no. 17000-10). DNA that has been eluted in sterile water should be stored at –65 to –90°C.

Ordering Information

Product	Contents	Cat. no.
MagAttract PowerClean DNA Kit (384)	For 384 preps: Automated removal of PCR inhibitors from previously purified DNA using magnetic bead technology	27900-4-KF
Related products		
MagAttract PowerSoil® DNA KF Kit (384)	For 384 preps: Hands-free isolation of DNA from soil using automated processing and liquid handling systems	27000-4-KF
MagAttract PowerSoil DNA EP Kit (384)	For 384 preps: Hands-free isolation of DNA from soil using automated processing and liquid handling systems	27100-4-EP
MagAttract PowerMicrobiome® DNA/RNA EP Kit (384)	For 384 preps: Hands-free isolation of nucleic acids from stool and gut material using an automated processing or liquid handling system	27500-4-EP
MagAttract PowerMicrobiome DNA/RNA KF Kit (384)	For 384 preps: Hands-free isolation of nucleic acids from stool and gut material using an automated processing or liquid handling system	27600-4-EP
MagAttract PowerMicrobiome DNA/RNA KF Kit (384)	For 384 preps: Hands-free isolation of nucleic acids from stool and gut material using an automated processing or liquid handling system	27600-4-KF

Product	Contents	Cat. no.
MagAttract PowerWater® DNA/RNA Kit (384)	For 384 preps: Automated isolation of nucleic acids from filtered air and water samples	27800-4-EP

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.

Limited License Agreement for MagAttract PowerClean DNA Kit

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