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MagAttract® Blood DNA/RNA Kit Handbook

For hands-free isolation of DNA from blood using a KingFisher™ Flex or Duo platform

Sample to Insight

QIAGEN

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Kit Contents

MagAttract Blood DNA/RNA Kit	**(384)**
Catalog no.	**22100-4-KF**
Number of preps	**4 x 96**
RBC Lysis Solution	255 ml
WBC Lysis Solution	220 ml
ClearMag® Binding Solution	200 ml
ClearMag Zorb Reagent	9 ml
ClearMag Wash Solution	2 x 320 ml
RNase-Free Water	50 ml
Collection Plates (2 ml)	4
Sealing Tape	16
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Storage

The MagAttract Blood DNA/RNA Kit reagents and components 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.**

WBC Lysis Solution 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 Blood DNA/RNA Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

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

The MagAttract Blood DNA/RNA Kit can be used for automated, high-throughput and simultaneous co-isolation of DNA and RNA from 200 µl of anti-coagulant (EDTA and heparin) stabilized whole blood. This protocol has been validated with fresh blood (including blood stored in commercial preservation solutions; see the Troubleshooting Guide) and can isolate DNA from buffy coats as well as frozen blood. Use of QIAGEN's ClearMag magnetic beads enables efficient co-extraction of DNA and RNA from the same sample, resulting in high yields, excellent purity and DNA of high molecular weight (up to 100 kb). ClearMag bead technology also prevents the co-isolation of common blood-derived PCR inhibitors, thereby ensuring successful downstream applications such as PCR and RT-PCR.

Principle and procedure

Whole or preserved blood is added to a 2 ml Collection Plate (provided). After aliquoting blood samples, the MagAttract Blood DNA/RNA Kit protocol begins with erythrocyte lysis using a hypotonic buffer that enables removal of red blood cells (RBCs). After centrifugation, nucleic acids are extracted from white blood cells (WBCs) in the pellet using a highly denaturing chaotropic buffer that stabilizes and protects nucleic acids from liberated nucleases.

After lysates are generated, they are combined with ClearMag Beads and transferred to the KingFisher instrument. The remaining protocol steps are fully automated, with DNA and RNA co-eluted in RNase-Free Water.

The order of placement of components and reagents for the platform portion of the protocol will be described in the downloaded software specific to the KingFisher platform being used.

The MagAttract Blood DNA/RNA 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 the manufacturer of your robot for help in adapting this protocol to that system.

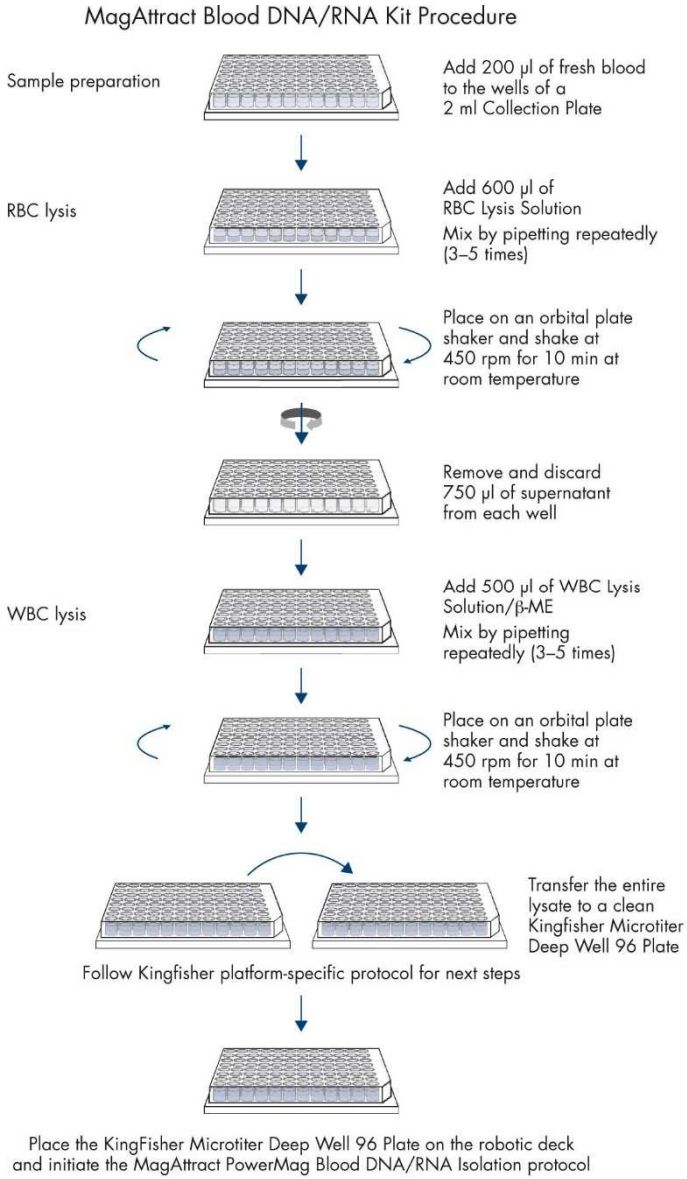

Figure 1. MagAttract Blood DNA/RNA Kit procedure.

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 6 cm) at 2000 x *g*
- Multi-channel pipettors (10–900 µl)
- Single-channel pipettors (10–1000 µl)
- Orbital plate shaker capable of 450 rpm
- β-Mercaptoethanol (β-ME); 480 µl of β-ME will be required for each 96 well plate
- 100% ethanol; 190 ml of 100% ethanol will be required for each 96 well plate
- Please contact your Thermo Scientific representative for specific KingFisher Flex and KingFisher Duo consumables.
- Multi-channel pipettor reagent reservoirs (5–300 ml)
- Appropriate tips for single-use and multi-channel pipettors to be used in the lysate preparation steps

Important Notes

- Blood samples may contain different numbers of WBCs, which affects the quantity of nucleic acids isolated from each sample. WBC counts can vary based on the health of the subject at the time of blood sampling. Nucleic acid yields vary by species as well.
- An important consideration for extracting RNA from blood is the handling and storage of samples. Blood should be collected in tubes containing an anticoagulant such as EDTA, ACD, CPD-A or heparin. Extractions should be performed as soon as possible (within 2 hours) after blood collection to obtain the highest quality and yields of DNA and RNA. Samples should not be frozen, particularly if they are to be used for applications where RNA is desired, unless validated DNA/RNA stabilizers, such as PAXgene® Tubes (**www.preanalytix.com**) or the Biomatrica® RNAgard® Blood System (**www.biomatrica.com/rnagardblood_tube.php**) is used. Always use fresh blood for optimal results.
- You will need 190 ml of 100% ethanol for each 96 well plate being processed on the KingFisher Flex. The KingFisher Duo will require 22 ml 100% ethanol per 12 wells to be processed.

Protocol

Important points before starting

- For each 96 well plate, add 500 µl of fresh β-Mercaptoethanol (β-ME) to 49.5 ml of WBC Lysis Solution (1% v/v; 1:100 dilution). Each individual sample requires 5 µl of β-ME and 495 µl of WBC Lysis Solution.
  Note: Prepare WBC Lysis Solution with fresh β-ME according to the number of samples being processed instead of adding β-ME to the whole bottle.

Procedure

1. Dispense 200 µl of well-mixed, fresh blood into each well of a 2 ml Collection Plate (for preserved blood, please see the Troubleshooting Guide).
2. Add 600 µl of RBC Lysis Solution to each well and mix the sample by repeated pipetting (3–5 times). Apply a piece of Sealing Tape (provided) to the top of the 96 well plate. Place on an orbital plate shaker and shake at 450 rpm for 10 min at room temperature.
3. Centrifuge the 2 ml Collection Plate at 2000 x *g* for 5 min to pellet intact white blood cells (WBCs). Remove Sealing Tape and discard.
   Note: Remember to use a balance plate for the centrifuge; use 800 µl of water per well.
4. Remove and discard 750 µl of RBC lysate from each well. We recommend inserting your pipette tip along the wall of the well to avoid disturbing the WBC pellet at the center of the well floor.
   Note: If using a digital pipettor to remove the RBC lysate, we recommend setting the aspiration speed to the lowest setting.
5. Add 500 µl of WBC Lysis Solution/β-ME to each well and resuspend the WBC pellet by repeated pipetting (3–5 times). Apply a new piece of Sealing Tape (provided).
6. Place on an orbital plate shaker and shake at 450 rpm for 10 min at room temperature. If isolating DNA and RNA on the KingFisher Flex, go to page 12. If using the KingFisher Duo, go to page 13.

KingFisher Flex

Continued from step 6 on page 11

7. Transfer the entire WBC lysate (from the 2 ml Collection Plate in step 6 on page 11) to the appropriate wells on a KingFisher Flex Microtiter Deepwell 96 plate (user supplied).
8. Resuspend ClearMag Beads (Zorb Reagent) by vortexing the bottle. For each plate to be processed, add 2 ml of the resuspended ClearMag Beads to 41 ml of ClearMag Binding Solution in an appropriate vessel (user provided) and immediately transfer to a multi-channel reservoir (user provided).
9. Mix the bead dispersion thoroughly and add 430 µl of the ClearMag Beads/ClearMag Binding Solution mixture to each well containing lysate.
   Note: Work quickly as the ClearMag Beads will slowly settle. It is important to maintain the beads in suspension to ensure uniform distribution into each well.
10. Place the KingFisher Flex Microtiter Deepwell 96 plate containing WBC lysate and ClearMag mixture on the deck as indicated in the display on the instrument.
11. You will need three KingFisher Flex Microtiter Deepwell 96 plates for this step. Place 900 µl of ClearMag Wash Solution into each well of one plate. Place 900 µl of 100% ethanol into each well of the other two plates. Place each plate on the deck as indicated in the display, with the ClearMag wash plate placed before the two ethanol wash plates.
12. Place 50–100 µl of RNase-Free Water into each corresponding well of a KingFisher 96 KF microplate and place on the deck as indicated.
13. Initiate the KingFisher PowerMag® Blood DNA/RNA Isolation protocol program.
14. Upon completion, cover the wells of the KingFisher 96 KF microplate with an appropriate storage seal (user provided). The DNA and RNA are now ready for downstream applications. We recommend storing eluents between –65°C and –90°C to maintain RNA integrity.

KingFisher Duo

Continued from step 6 on page 11

7. Transfer the entire WBC lysate from up to 12 wells of the 2 ml Collection Plate (from step 6 on page 11) to the first long row (A) of a KingFisher Flex Microtiter Deepwell 96 plate (user provided).
   Note: The Kingfisher Duo limits the number of samples that can be processed at one time. If processing more than 12 samples, you may place any additional sample lysates in the 2 ml Collection Plate at 2–8°C until they are ready to be processed.
8. Resuspend ClearMag Beads (Zorb Reagent) by vortexing the bottle. For each set of 12 wells to be processed, combine 5.74 ml of ClearMag Binding Solution and 280 µl of ClearMag Beads in an appropriate vessel (user provided). Mix well to obtain a homogeneous dispersion of beads.
9. Add 430 µl of the ClearMag Beads/Binding Solution to each well in Row A of the KingFisher Flex Microtiter Deepwell 96 plate from step 7.
   Note: Work quickly as the ClearMag Beads will slowly settle. It is important to maintain the beads in suspension to ensure uniform distribution into each well.
10. Place a KingFisher Duo 12-tip comb (user provided) into the second row (B) of the KingFisher Flex Microtiter Deepwell 96 Plate.
11. Add 900 µl of ClearMag Wash Solution to each well in row C of the of the KingFisher Flex Microtiter Deepwell 96 plate. Add 900 µl of 100% ethanol to rows D and E of the KingFisher Flex Microtiter Deepwell 96 plate and place on the deck.
12. Add 50–100 µl of RNase-Free Water to each well of a KingFisher Duo Elution Strip (user provided) and place the strip on the deck.
13. Initiate the KingFisher PowerMag Blood DNA/RNA Isolation protocol program.
14. Upon completion, cover the wells of the KingFisher 96 KF microplate with an appropriate storage seal (user provided). The DNA and RNA are now ready for downstream applications. We recommend storing eluents between –65°C and –90°C to maintain RNA integrity.

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)	Blood preservation methods	There is no change in protocol when using preserved blood but keep in mind that preserved blood samples will have been diluted with preservation solution. Do not freeze blood sample, unless a validated DNA/RNA stabilizer, such as the ones listed below, are used. PAXgene Tubes (**www.preanalytix.com**) Biomatrica RNAgard Blood System (**www.biomatrica.com/rnagardblood_tube.php**) Use the manufacturer's protocol and recommendations for preservation of whole blood.
DNA/RNA		
a)	DNA/RNA does not amplify	Make sure to check DNA/RNA yields by gel electrophoresis or spectrophotometer reading. Typically, 10 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 will still not amplify after altering the amount of template per reaction, PCR optimization (changing reaction conditions and primer choice) may be needed.

		Comments and suggestions
b)	Concentrating eluted DNA/RNA	The final volume of eluted nucleic acids will be 50–100 µl. The nucleic acids may be concentrated by adding 5 µl of 3 M NaCl and inverting 3–5 times to mix. Next, add 200 µl of 100% cold ethanol and invert 3–5 times to mix. Incubate at –15 to –30°C for at least 10 minutes to overnight for DNA and overnight at –15 to –30°C for RNA (for optimal recovery). 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 RNase-Free Water or 10 mM Tris. **Note:** This procedure must be done after transferring the eluted samples to individual microcentrifuge tubes.
c)	Storing DNA/RNA	Nucleic acids are eluted in RNase-Free Water and should be stored at –65 to –90°C to prevent degradation. DNA and RNA can be eluted in 10 mM Tris (pH 7.0) or TE without loss, but EDTA may inhibit downstream applications such as PCR and automated sequencing. Prolonged storage in the microplates at 2–8°C will result in the loss of liquid due to evaporation.

Ordering Information

Product	Contents	Cat. no.
MagAttract Blood DNA/RNA Kit (384)	For 384 preps: Hands-free isolation of DNA from blood using a KingFisher Flex or Duo	22100-4-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 Microbial DNA Kit (384)	For 384 preps: Isolation of DNA from microbial and food cultures using automated processing and liquid handling systems	27200-4
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 PowerWater® DNA/RNA Kit (384)	For 384 preps: Automated isolation of nucleic acids from filtered air and water samples	27800-4-EP
MagAttract PowerClean® DNA Kit (384)	For 384 preps: Automated removal of PCR inhibitors from previously purified DNA using magnetic bead technology	27900-4-KF

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

Limited License Agreement for MagAttract Blood DNA/RNA 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.
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