



Promega

Technical Bulletin

MagneSil® KF, Genomic System

INSTRUCTIONS FOR USE OF PRODUCT MD1460.



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MagneSil® KF, Genomic System

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Technical Bulletin. Please contact Promega Technical Services if you have questions on use
of this system. E-mail techserv@promega.com.

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1. Description

Traditional procedures for isolating genomic DNA from blood and other samples can be lengthy and potentially hazardous. The extensive centrifugation and incubation steps used in some procedures may make it impossible to complete DNA purification in one day. In the past, automated methods and instrumentation have been expensive and available only for high-throughput applications. The MagneSil® KF, Genomic System^(a) is designed to efficiently purify 2–4µg of gDNA from 200µl of anti-coagulated whole blood depending upon the white cell count and is used in conjunction with automated protocols on the Thermo Electron KingFisher® mL magnetic particle processor instrument. The purification protocol can be obtained from Promega.

The MagneSil® KF, Genomic System has sufficient reagents for 200 standard DNA purifications from 200µl whole blood each. Other samples such as stool, tissue, blood punches and other liquid materials may be used with some additional processing prior to using the KingFisher® mL instrument. Please contact Promega or visit our web site for the latest information and protocols.

The KingFisher® mL instrument can process simultaneously one to fifteen samples using a 25-minute, walkaway method. Two specialized disposables are required; a 1 × 5 tube strip and a tip comb. Each purification uses one 1 × 5 tube strip per sample. The tip comb is designed with 5 tips to purify up to 5 samples. Prefill the 1 × 5 tube strip with reagents, add sample, place the tube strip and tip comb into the KingFisher® mL instrument, select the protocol and press start to begin purification.

DNA purified with the MagneSil® KF, Genomic System can be used in fluorescent STR and PCR as well as more stringent applications such as multiplexed PCR (e.g., the Y Chromosome Deletion Detection System, Version 2.0 [Cat.# MD1531]) or the READIT® SNP Genotyping System [Cat.# MD1290]).

2. Product Components and Storage Conditions

Product	Size	Cat.#
MagneSil® KF, Genomic System	200 preparations	MD1460

The MagneSil® KF, Genomic System contains sufficient reagents to perform 200 purifications from 200µl of blood. Includes:

- 40ml MagneSil® KF Paramagnetic Particles
- 160ml Lysis Buffer, KF
- 200ml Salt Wash, Blood
- 220ml Alcohol Wash, Blood
- 2 × 25ml Nuclease-Free Water

Storage Conditions: Store at 22–25°C. Do not freeze the MagneSil® KF Particles.

3. Initial Preparation of the KingFisher® mL Instrument

3.A. Hardware Setup

The plastic tip comb should be inserted into the instrument in such a way that the magnets line up along the middle of each tip in the tip comb. Ensure that the tip comb is properly in place by pushing the tip comb until it “snaps” into position. If the tip combs are not fully seated and aligned correctly, the KingFisher® mL instrument will give you a “head alignment error” message when you start the run (see Section 5). If this happens, reposition the tip comb so it’s centered, clear the machine by pressing stop, and then restart the program.

3.B. Installation

Equipment to Be Supplied By the User

(For more information about Thermo Electron products, please contact your local Thermo Electron representative)

- KingFisher® mL Instrument (Thermo Electron Part# 5400050)
- laptop or desktop computer with a minimum of Windows® 95 operating system
- serial connection cable to connect the computer to the KingFisher® instrument Fq/FI5 serial cable (for RS-232 port; Thermo Electron Part# 2305290)
- 1 × 5 tube strips (Thermo Electron Part# 97002121)

Software to Be Supplied By the User

- Hyperterminal program (supplied with Windows® operating systems)

Hyperterminal Program Settings

To use the MagneSil® KF System protocol on the KingFisher® mL instrument, you must first install the protocol onto the instrument from a computer. The transfer of the protocol is performed using the Hyperterminal program, an executing program that transfers text files from the computer to the KingFisher® mL instrument. It is part of the operating system of most Windows®-based computers.

Note: The program for the MagneSil® KF System is available to download at: www.promega.com/automethods/

The Hyperterminal program is located under the “Start” button in: Programs/Accessories/Communications/Hyperterminal

The Connection Description window is the window that first appears when you open the program.

1. Enter “Hyper” in the Name Field and then click “ok”. The Connect To window will appear.
2. In the Connect To window, select “connect using com 1”, or the appropriate Com setting (e.g., com 2) from the drop down menu and then click “ok”. The Hyperterminal Program settings window will appear.
3. **Hyperterminal Program Settings Window:** Connect using Com 1.

Com 1 properties	
Bits per sec	9600
Data Bits	8
Parity	None
Stop Bits	1
Flow Control	Xon/Xoff

4. Click “ok”.

Loading the KingFisher® Protocol

The KingFisher® protocol is a simple text file. The file name will end with a .txt extension. The protocol for the MagneSil® KF System is available to download at www.promega.com/automethods/

1. After selecting the settings of the Hyperterminal program as described, download the KingFisher® mL protocol (the text file) onto the KingFisher® mL instrument.
2. Under the Transfer menu, select "Send Text file".
3. A browse window will appear. Browse to the location of the KingFisher® mL text file. Select the KingFisher® mL text file "KfmLMagGenom1_0".
4. Lines of text will scroll by on the computer screen. The KingFisher® mL instrument will read "Computer Control".
5. Once the file has been transferred to the KingFisher® mL instrument, the text on the computer screen will read "PAL". The PAL will continue to appear. This means that the program has been transferred.
6. Exit out of the Hyperterminal program.
7. Push "Stop" on the KingFisher® mL instrument to end Computer Control of the Instrument.
8. Scroll through the programs on the KingFisher® mL instrument. You should see the protocol listed as "PromegaGenomic".

4. Before You Begin

Reagents to Be Supplied By the User

- 95-100% ethanol
- isopropanol

Equipment to Be Supplied By the User

(For more information about Thermo Electron products, please contact your local Thermo Electron representative)

- tip combs (Thermo Electron Part# 97002111)
- KingFisher® mL 1 × 5 tube strip (Thermo Electron Part# 97002121)

4.A. Preparation of Alcohol Wash, Blood

Add recommended amount of isopropanol and ethanol indicated on the label to the bottle **before first use** and mix well to prepare the working solution. Store the bottle tightly capped to minimize alcohol evaporation.

4.B. Preparation of the 1 × 5 Tube Strip for gDNA Purification from Blood

Mix blood samples that have been stored by inverting the tube until the blood returns to a homogeneous solution. Blood may be collected under EDTA, ACD heparin or citrate anti-coagulants. It is easier to add all of the solutions in tubes 1-5 first, followed by the blood, thus requiring less handling of blood.

! Mix the MagneSil® KF Particles vigorously to ensure complete resuspension of the particles prior to removing an aliquot.

Assemble the 1 × 5 tube strip as follows:

1. Starting with the left-most tube: Lysis Buffer, KF (800µl) + 200µl of MagneSil® KF Particles, + 200µl of blood.
2. Second tube from left: 1,000µl of Salt Wash, Blood.
3. Third and fourth tube from left: 1,000µl of Alcohol Wash, Blood.
4. Right-most tube: 200µl of Nuclease-Free Water (this results in a final recovered volume of about 190µl).

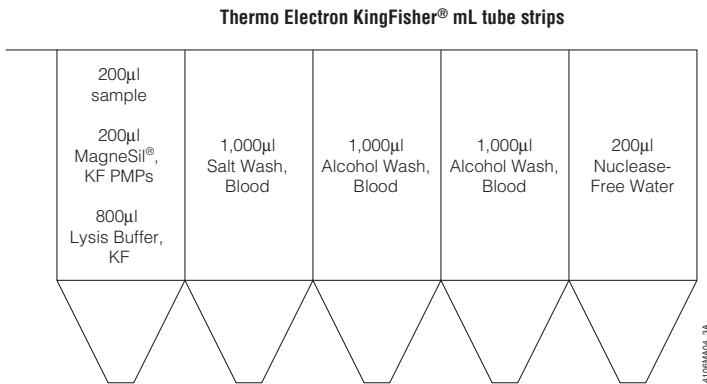


Figure 1. The MagneSil® KF, Genomic System assembly of the KingFisher® mL tube strip. Volumes indicated are for genomic DNA Purification from 200µl of whole blood.

5. Performing the Method

Select the MagneSil® KF method “PromegaGenomic” by scrolling through the installed methods using the arrow keys on the control pad. Check that the magnet tip comb(s) is in place and the tube strips in the stainless steel rack are properly inserted into the housing. Press the start button on the instrument. You may close the hinged doors on the top and the front of the instrument or leave them open.

To end the program, press stop twice, and the head will return to its original up/left position.

When the run is complete, the unit will beep. Push stop to end the beeping. We recommend performing a "dry run" with the plastics but with no samples before your first actual use of the system.

6. Description of Method

This overview describes the particle handling steps required for automated use of the MagneSil® KF, Genomic System on the Thermo Electron KingFisher® mL Instrument. The KingFisher® mL processes up to fifteen samples. Each sample uses a plastic tube strip containing 5 wells and a tip-comb:

1. **Lysis and DNA Binding.** In the first well the sample is lysed and the DNA is bound to MagneSil® KF paramagnetic particles.
2. **Salt Wash.** The MagneSil® KF paramagnetic particles are transferred to the second well where they are washed with a salt wash.
3. **Alcohol Wash.** The MagneSil® KF paramagnetic particles are transferred to the third well for the first of two alcohol washes. The second alcohol wash occurs when The MagneSil® KF paramagnetic particles are transferred to the fourth well.
4. **Particle drying.** The particles are suspended above the fourth well and air dried.
5. **Elution.** The DNA is eluted in water in the fifth well. Following elution, the particles are removed to well 4, leaving the eluted DNA ready for PCR or other analysis.

7. Determining Yield

The eluted DNA will be in about 200µl of water (approximately 10µl stays with the previously dried particles).

Estimate the DNA yield using PicoGreen® stain or spectrophotometry and the following formula:

$A_{260} - A_{320}$ = corrected A_{260} to be used in calculation to estimate yield

$A_{260} - A_{320} / A_{280} - A_{320}$ = estimate of DNA purity

Note: A_{320} measures light scattering. A high A_{320} reading results in artificially high estimates of yield (A_{260}) but also reduces estimated DNA purity (A_{260} / A_{280}).

8. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor.
 Contact information available at: www.promega.com E-mail: techserv@promega.com

Symptoms	Causes and Comments
Low DNA yields	<p>Amount of MagneSil® KF Particles may need to be optimized. Using 250µl of MagneSil® KF PMPs rather than the 200µl specified in the protocol may increase the DNA yields for samples that have a high amount of white cells in the blood sample but may reduce the yields for samples with low white counts. The surface area available for binding DNA is increased when 250µl is used. However, the efficiency of the washes is reduced, since there is additional surface area requiring cleaning. For samples with less DNA (and lower white cell counts), the increased binding area may not increase DNA yields, since there is sufficient surface available for DNA binding already. With the efficiency of the washes reduced, the yields for these lower white cell samples may be reduced. For these samples, use less than 200µl of MagneSil® KF PMPs.</p>
Inconsistent yields for the same sample across different locations	<p>Stainless steel tray is not level. Leveling the tray under the sample locations where yields are lower; the sample tray should produce more consistent yields from the same sample across the plate.</p> <p>Variability in white blood cell counts in the blood sample. In general, there will be variability in yields due to differences in white blood cell counts.</p>
DNA does not work in PCR or other downstream applications	<p>Residual alcohol carried over in the elution. Allow the eluted DNA to air-dry for 30 minutes at room temperature so that any residual alcohol can evaporate. Try the PCR again. Vary the amount of template to see if more or less template allows for successful PCR.</p>
Eluted DNA appears colored (brown or pale yellow)	<p>MagneSil® particle carryover. MagneSil® PMP carryover may make the sample appear brownish or cloudy. If this occurs, MagneSil® PMPs can be removed by gravity settling, by placing sample near a magnet or by brief centrifugation. The presence of small amounts of PMPs generally has no effect on amplification reactions.</p>

8. Troubleshooting (continued)

Symptoms	Causes and Comments
Eluted DNA appears colored (brown or pale yellow) (continued)	Heme carryover. Occasionally DNA may appear a pale yellow due to heme carryover. This pale yellow generally has no impact on amplification reactions or other downstream applications.
Head alignment error on the machine	Plastic sleeve not properly inserted into the designated slot. If the sleeve is not properly inserted, the magnets are unable to be inserted inside the plastic sleeve. For other errors, please see the instrument instruction manual.
Hyperterminal errors when downloading the protocol into the KingFisher® mL	Incorrect com port designated; com port in use or assigned. Be sure the correct com port (e.g., com 1 or com 2) is being used. Disable the PDA or other device software that may use the com port.
The instrument display shows an error in downloading a method	KingFisher® mL instrument memory is full. Delete some files from the KingFisher® mL memory. For guidance, contact Promega Automation Support at techserv@promega.com The *.txt method has been modified. Contact Promega for the original method.

9. Related Products

Product	Size	Cat.#
MagneSil® KF, Paramagnetic Particles	40ml	MD1471
Lysis Buffer, KF	160ml	MD1521
Salt Wash, Blood	200ml	MD1401
Alcohol Wash, Blood	200ml	MD1412
Nuclease-Free Water	50ml (2 × 25ml)	P1193
PCR Master Mix*	10 reactions	M7501
	100 reactions	M7502
	1,000 reactions	M7505
GoTaq® DNA Polymerase*	100u	M3001
	500u	M3005
	2,500u	M3008
ART® 20P Pipet Tip, 20µl	960/pk	DY1071
ART® 100 Pipet Tip, 100µl	960/pk	DY1101
ART® 100E Pipet Tip, 100µl	960/pk	DY1111
ART® 200 Pipet Tip, 200µl	960/pk	DY1121
ART® 1000 Pipet Tip, 1,000µl	768/pk	DY1131
Y Chromosome Deletion Detection System, Version 2.0**	25 reactions	MD1531
READIT® SNP Genotyping System**	100 reactions	MD1290

* For Laboratory Use.

** For Research Use Only. Not for use in diagnostic procedures.

^(a)U.S. Pat. Nos. 6,027,945 and 6,368,800, Australian Pat. No. 732756, Japanese Pat. No. 3253638, European Pat. No. 0 895 546 and Mexican Pat. No. 209436 have been issued to Promega Corporation for methods of isolating biological target materials using silica magnetic particles. Other patents are pending.

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