

MagAttract[®] Direct mRNA M48 Handbook

For efficient, low- to medium-throughput preparation of mRNA from whole blood and cultured cells using the BioRobot[®] M48 workstation



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Kit Contents

MagAttract Direct mRNA M48 Kit	(192)
Catalog no.	957336
Number of preps	192
MagAttract Suspension C	10 ml
Buffer MRL	90 ml
Buffer MRW1	110 ml
Buffer MRW2	160 ml
Buffer MRE	15 ml
Handbook	1

Storage

All buffers and reagents should be stored at 2–8°C.

Product Use Limitations

The MagAttract Direct mRNA M48 Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN® product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover).

Safety Information

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

If liquid containing potentially infectious agents is spilled on the BioRobot M48, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite, followed by water.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the MagAttract Direct mRNA M48 Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

Quality Control

As part of the stringent QIAGEN quality assurance program, the performance of MagAttract Direct mRNA M48 Kits is monitored routinely and on a lot-to-lot basis. All kit components are tested separately to ensure highest performance and reliability.

Introduction

This handbook provides a guide to automated purification of mRNA from whole blood or cultured cells using the MagAttract Direct mRNA M48 Kit in combination with the BioRobot M48 workstation.

The MagAttract Direct mRNA M48 Kit is designed for automated purification of mRNA from pretreated whole blood and cultured cell lysates. MagAttract magnetic particle technology provides high-quality mRNA that is suitable for direct use in downstream applications, such as amplification or other enzymatic reactions. The BioRobot M48 performs all steps of the purification procedure, which can be scaled up or down, allowing purification from varying amounts of starting material. This handbook contains protocols for purification of mRNA from pretreated human whole blood and from cultured cells.

Supplementary protocols for automated purification of mRNA from other sample types using the MagAttract Direct mRNA M48 system will be available at www.qiagen.com/goto/clinicalRNA.

Principle and procedure

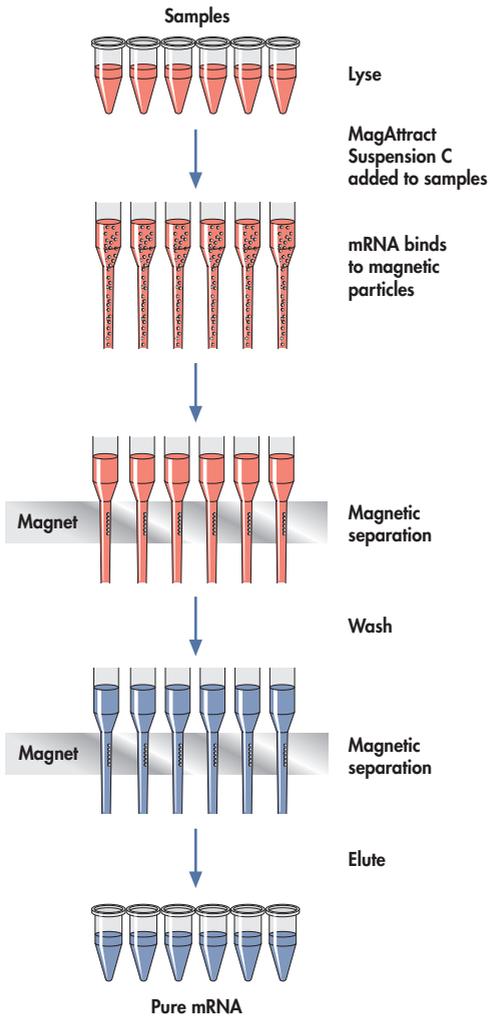
MagAttract Direct mRNA technology combines the convenient handling of magnetic particles with the specificity of oligo-dT hybridization. Protocols require short pretreatment steps.

Oligo-dT probes are covalently attached to the surface of MagAttract Direct mRNA particles. mRNA binds rapidly and efficiently to the oligo-dT probes on the MagAttract Direct mRNA particles in the presence of Buffer MRL (see flowchart). mRNA bound to the magnetic particles is then efficiently washed. Two different wash buffers are used to considerably improve the purity of the mRNA. Highly pure mRNA is eluted in the elution buffer (Buffer MRE) provided. mRNA yields depend on sample type, sample storage, and white blood cell content (for whole blood samples).

Software

The QIAsoft M Operating System provides an easy to use interface for controlling the BioRobot M48 workstation. Sample information and the number of samples are entered into prepared fields. The number of disposable filter-tips required for the run are calculated by the QIAsoft M Operating System and added to the worktable by the user. All subsequent steps are performed automatically.

MagAttract Direct mRNA M48 Procedure



Starting material

The amounts of starting material for use in MagAttract Direct mRNA M48 procedures are shown in Table 1. The MagAttract Direct mRNA M48 Kit is optimized for use with 150–740 μl sample lysates. The sample and elution volumes for each protocol can be scaled within the ranges shown to give a yield and concentration of high-quality mRNA appropriate for the intended downstream application.

mRNA purification is performed from lysates of up to 2×10^6 cultured cells or pretreated blood cells (leukocytes). Elution volumes can be scaled to the requirements of the user (50–100 μl), but the protocols are optimized for 100 μl . Elution in smaller volumes will increase the final mRNA concentration in the eluate but slightly reduce the overall mRNA yield.

Table 1. Sample and Elution Volumes Used in MagAttract Direct mRNA M48 Procedures

Protocol	Standard	Standard	Standard	Double Scale
Sample volume	150 μl	250 μl	370 μl	740 μl
Number of cells	$<4 \times 10^5$	$<7 \times 10^5$	$<1 \times 10^6$	$<2 \times 10^6$
Elution volume	50–100 μl	50–100 μl	50–100 μl	50–100 μl

Storage of blood samples

Fresh whole blood samples treated with EDTA, ACD (citrate), or heparin can be used to prepare leukocytes. Leukocyte samples may then be frozen at -70°C . Yield and quality of the purified mRNA depends on the sample storage conditions used. Fresher blood samples may yield better results.

Yield of purified mRNA

mRNA yields depend on the sample type and condition, number of actively transcribing cells in the sample, and the protocol used for isolation of mRNA. Typically, mRNA isolation from 2×10^6 cultured BJAB cells gives a yield of 2 μg mRNA.

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 material safety data sheets (MSDSs), available from the product supplier.

- BioRobot M48 workstation
- Filter-Tips, 1000 µl, M48 (1000), cat. no. 995652
- Reagent Containers, small, M48 (100), cat. no. 995902
- Reagent Containers, large, M48 (50), cat. no. 995904
- Reagent Container Seals, M48 (50), cat. no. 995906
- Sample Prep Plates, 42-well, M48 (100), cat. no. 995908
- Sample and elution tubes with screw caps, 1.5 ml (Sarstedt, cat. no. 72.692)*†

Optional:

- Cooling Block, 48-tube, 0.2 ml, M48, cat. no. 9015178
- Cooling Block, 48-tube, 1.4 ml, M48, cat. no. 9015180

* This is not a complete list of suppliers and does not include many important vendors of biological supplies; however, use of other tubes may result in an instrument crash.

† mRNA can also be eluted into 0.2 ml thin-walled PCR tubes or 1.4 ml tubes.

Important Notes

Buffer MRL

Before use, check that Buffer MRL does not contain a white precipitate by shaking the bottle. Check again when pipetting Buffer MRL into the reagent container. If necessary, incubate for 15 minutes at 37°C with occasional shaking to dissolve the precipitate.

MagAttract Suspension C

Shake the bottle containing MagAttract Suspension C and vortex for 3 minutes (before first use) or 1 minute (before subsequent uses) to ensure that the magnetic particles are fully resuspended.

Residual reagents

Residual reagents should either be removed immediately from the workstation and transferred to an airtight container or discarded. Residual Buffer MRL should always be discarded.

Quantification of mRNA

Carryover of MagAttract particles may affect the absorbance reading at 260 nm (A_{260}) of the purified mRNA but should not affect downstream applications. To correct for any background absorbance, subtract the absorbance value measured at 320 nm from measurements at 260 nm and 280 nm. See "Quantification of mRNA", Appendix, page 16 for more information.

Protocol: Purification of mRNA from Cultured Cells

Things to do before starting

- Before beginning the procedure, read “Important Notes” on page 10.

Procedure

1. **Place suspended cells (up to 1×10^6 for the mRNA Standard protocol and up to 2×10^6 for the mRNA Double Scale protocol. See Table 1, page 8) in a 1.5 ml microtube and centrifuge for 5 min at $300 \times g$. Discard the supernatant and wash the cell pellet twice using $1 \times$ PBS.**

Samples may then be frozen and stored at -70°C , or used immediately.

2. **Resuspend the cell pellet in the appropriate volume of Buffer MRL to attain the required sample volume (see Table 1, page 8). Lyse cells quickly by pipetting up and down several times through a 200 μl pipet tip.**
3. **The viscosity of 700–740 μl lysates (Double Scale protocol) should be reduced by a DNA-shearing step using QIAshredder homogenizers or the TissueLyser.**

Alternatively, lysates can be homogenized by forcibly passing them 3 times through a 23-gauge needle fitted to an RNase-free syringe. Reduce any foam that may form by centrifuging the tube for 30 s at $300 \times g$.

Note: Lysates less than 370 μl (Standard protocol) do not require a DNA-shearing step.

4. **Ensure that the BioRobot M48 is switched on.**

The power switch is on the left side of the instrument.

5. **Switch on the computer and monitor.**

6. **Launch the QIAsoft M Operating System.**

Upon startup, the computer controlling the BioRobot M48 is normally set to launch the QIAsoft M software startup window, but this setting may have been changed.

The QIAsoft M Operating System can also be started from the QIAsoft M icon on the desktop or from the Microsoft® Windows® “Start” menu, where it is located in QIAsoft M Operating System → QIAsoft M V2.0 for BioRobot M48.

7. **Select the desired protocol (“MagAttract mRNA Standard” or “MagAttract mRNA Double Scale”) from the drop-down menu by clicking on the dark green arrow.**
8. **Click the “Select” button to choose the elution tube type. Enter the number of samples and the sample and elution volumes into the software.**

The QIAsoft M software will now guide you through the remaining steps required to set up the BioRobot M48 for the chosen protocol; these steps include the option of entering names for your samples. Follow the steps detailed in each protocol message before continuing. Wear gloves when loading the required items on the worktable.

9. **Close the workstation door and start the purification protocol. All steps are fully automated, and a software message on the screen will indicate when the protocol is finished.**
10. **Retrieve the elution tubes containing the purified mRNA from the cooling block. The mRNA is ready to use, or can be stored at -20°C for 24 h or at -70°C for longer periods.**

Carryover of magnetic particles in eluates will not affect most downstream applications. If removal of magnetic particles is required, immobilize particles using a suitable magnetic separator and transfer the eluate to a clean tube (see Appendix, page 16). The risk of magnetic-particle carryover is minimal.

Protocol: Purification of mRNA from Whole Blood

Things to do before starting

- Before beginning the procedure, read “Important Notes” on page 10.
- mRNA isolation and purification from blood is performed after an initial preparation of leukocytes (white blood cells). Such preparation methods can be found in published literature (e.g., Bøyum, A. [1968] *Separation of leukocytes from blood and bone marrow. Scand. J. Clin. Invest.* **21**, suppl. 97) or purchased as kits (Lymphoprep™, product number 1031966, AXIS-SHIELD).
- The number of leukocytes in blood may vary considerably ($2\text{--}10 \times 10^6$ per ml of blood). The number of cells will be reflected in the yield of mRNA. Choose the sample volume appropriate for the numbers of cells in the sample and for the downstream application (see “Starting material”, page 8).

Procedure

1. **Prepare leukocytes using one of the methods recommended above (see “Things to do before starting”) and pellet cells by centrifuging for 5 min at 3000 x g.**

The sample may be processed immediately, using the procedure below, or frozen at -70°C for later use.

Note: Quantify prepared cells using a hemocytometer. The Standard protocol is designed for isolation of mRNA from lysates of up to 1×10^6 cells, while the Double Scale protocol is for isolation of mRNA from up to 2×10^6 cells.

2. **Resuspend the cell pellet in the appropriate volume of Buffer MRL to attain the required sample volume (see Table 1, page 8). Lyse cells quickly by pipetting up and down several times through a 200 μl pipet tip.**
3. **The viscosity of 700–740 μl lysates (Double Scale protocol) should be reduced by a DNA-shearing step using QIAshredder homogenizers or the TissueLyser.**

Alternatively, lysates can be homogenized by forcibly passing them 3 times through a 23-gauge needle fitted to an RNase-free syringe. Reduce any foam that may form by centrifuging the tube for 30 s at $300 \times g$.

Note: Lysates less than 370 μl (Standard protocol) do not require a DNA-shearing step.

4. **Depending on the number of cells in the starting material, transfer 150 μl , 250 μl , or 370 μl supernatant into a 1.5 ml sample tube if using the Standard protocol or transfer 740 μl supernatant into a 2 ml sample tube if using the Double Scale protocol (See Table 1, page 8 for details).**
5. **Ensure that the BioRobot M48 is switched on.**
The power switch is on the left side of the instrument.
6. **Switch on the computer and monitor.**

7. Launch the QIAsoft M Operating System.

Upon startup, the computer controlling the BioRobot M48 is normally set to launch the QIAsoft M software startup window, but this setting may have been changed.

The QIAsoft M Operating System can also be started from the QIAsoft M icon on the desktop or from the Microsoft Windows "Start" menu, where it is located in QIAsoft M Operating System → QIAsoft M V2.0 for BioRobot M48.

8. Select the desired protocol ("MagAttract mRNA Standard" or "MagAttract mRNA Double Scale") from the drop-down menu by clicking on the dark green arrow.**9. Click the "Select" button to choose the elution tube type. Enter the number of samples and the sample and elution volumes into the software.**

The QIAsoft M software will now guide you through the remaining steps required to set up the BioRobot M48 for the chosen protocol; these steps include the option of entering names for your samples. Follow the steps detailed in each protocol message before continuing. Wear gloves when loading the required items on the worktable.

10. Close the workstation door and start the purification protocol. All steps are fully automated, and a software message on the screen will indicate when the protocol is finished.**11. Retrieve the elution tubes containing the purified mRNA from the cooling block. The mRNA is ready to use, or can be stored at -20°C for 24 h or at -70°C for longer periods.**

Carryover of magnetic particles in eluates will not affect most downstream applications. If removal of magnetic particles is required, immobilize particles using a suitable magnetic separator and transfer the eluate to a clean tube (see Appendix, page 16). The risk of magnetic-particle carryover is minimal.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or molecular biology applications (see back cover for contact information).

Comments and suggestions

General handling

QIAsoft M software error dialog box If the QIAsoft M software displays an error dialog box during a protocol run, refer to the Troubleshooting Guide in the *BioRobot M48 User Manual*.

Low mRNA yield

- a) Incomplete sample lysis Before use, check that Buffer MRL does not contain a white precipitate by shaking the bottle. Check again when pipetting Buffer MRL into the Reagent Container. If necessary, incubate for 15 minutes at 37°C with occasional shaking to dissolve the precipitate.
- b) MagAttract Suspension C was not completely resuspended Before starting the procedure, ensure that the MagAttract Suspension C is fully resuspended. Vortex for at least 3 minutes before first use, and for 1 minute before subsequent uses.
- c) Reagents were loaded onto worktable in wrong order Ensure that all reagents were loaded onto the worktable in the correct order. Repeat the purification procedure with new samples.

mRNA does not perform well in downstream applications

- a) Contamination with genomic DNA Ensure the sample lysate has undergone a DNA-shearing step.
- b) Insufficient mRNA used in downstream application Quantify the purified mRNA by spectrophotometric measurement of the absorbance at 260 nm (see “Quantification of mRNA”, Appendix, page 16).
- c) Excess mRNA used in downstream application Excess mRNA can inhibit some enzymatic reactions. Quantify the purified mRNA by spectrophotometric measurement of the absorbance at 260 nm (see “Quantification of mRNA”, Appendix, page 16).

Appendix: Storage, Quantification, and Determination of Purity of mRNA

Storage of mRNA

Purified mRNA may be stored at -20°C for 24 hours or at -70°C for longer storage.

Quantification of mRNA

The concentration of mRNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. An absorbance of 1 unit at 260 nm corresponds to 44 μg of RNA per ml ($A_{260}=1 \rightarrow 44 \mu\text{g/ml}$). Use RNase-free buffer of neutral pH to dilute the samples and to calibrate the spectrophotometer. The ratio between the absorbance values at 260 nm and 280 nm gives an estimate of mRNA purity (see "Purity of mRNA", below). Carryover of magnetic particles in the eluate may affect the A_{260} reading, but should not affect the performance of the mRNA in downstream applications. If residual magnetic particles are to be removed, the tube containing the eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see below).

To quantify mRNA isolated using the MagAttract Direct mRNA M48 System:

- Apply the tube containing the mRNA to a suitable magnetic separator (e.g., QIAGEN 12-Tube Magnet, cat. no. 36912) for 1 minute.
- Once separation is complete, carefully withdraw 10–50 μl of purified mRNA and dilute to a final volume of 100 μl in buffer of neutral pH.
- If a suitable magnetic separator is not available, centrifuge the tube containing the mRNA for 1 minute at full speed in a microcentrifuge to pellet any remaining magnetic particles.
- Measure the absorbance at 320 nm, 280 nm, and 260 nm. Subtract the absorbance reading obtained at 320 nm from the readings obtained at 260 nm and 280 nm to correct for the presence of magnetic particles.

Concentration of mRNA sample = $44 \mu\text{g/ml} \times (A_{260} - A_{320}) \times \text{dilution factor}$

Total amount of mRNA isolated = concentration \times volume of sample in ml

Purity of mRNA

Purity is determined by calculating the ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm i.e., $(A_{260} - A_{320}) / (A_{280} - A_{320})$. Pure mRNA has an A_{260}/A_{280} ratio of 1.9–2.1 in 10 mM Tris-Cl, pH 7.5.

Ordering Information

Product	Contents	Cat. no.
MagAttract Direct mRNA M48 Kit (192)	MagAttract Suspension C and buffers for up to 192 preps	957336
BioRobot M48*	Robotic workstation for automated purification of nucleic acids using MagAttract magnetic particle technology	9000708
Accessories		
Starter Pack, M48	Pack includes: sterile filter-tips (600); sample preparation plates (40); large reagent containers (8); small reagent containers (8); silicon seals (8); sample tubes, 1.5 ml (250); sample tubes, 2 ml (250); elution tubes, screw cap, 1.5 ml (250); tip waste bags (2)	995999
Filter-Tips, 1000 µl, M48 (1000)	Sterile, disposable filter-tips, bagged; pack of 1000	995652
Reagent Containers, small, M48 (100)	Reagent Containers (20 ml) with lids. To be used with the Reagent Container Rack, M48. Pack of 100.	995902
Reagent Containers, large, M48 (50)	Reagent Containers (100 ml) with lids. To be used with the Reagent Container Rack, M48. Pack of 50.	995904
Reagent Container Seals, M48 (50)	Lid-sealing sheets for small and large Reagent Holders, allowing storage of unused reagents; pack of 50	995906
Sample Prep Plates, 42-well, M48 (100)	Disposable polypropylene plates for sample preparation, including nucleic acid binding and washing steps; pack of 100	995908
12-Tube Magnet	Magnet for separating magnetic particles in 12 x 1.5 ml or 2 ml tubes	36912

* QIAGEN Robotic Systems are not available in all countries; please inquire

Ordering Information

Product	Contents	Cat. no.
Related products		
PAXgene Blood RNA Tubes (100)	100 Blood Collection Tubes. To be used in conjunction with the PAXgene Blood RNA Kit (50).	762115
MagAttract RNA Cell Mini M48 Kit (192)	MagAttract Suspension E, Buffers, 4 x RNase-free DNase set, RNase-free water.	958336
MagAttract RNA Tissue Mini M48 Kit (192)	MagAttract Suspension E, Buffers, 4 x RNase-free DNase set, RNase-free water.	959336
Cooling Block, 48-tube, 0.2 ml, M48	Holder for accommodating 48 x 0.2 ml PCR tubes on the cooling and heating system of the BioRobot M48 worktable	9015178
Cooling Block, 48-tube, 1.4 ml, M48	Holder for accommodating 48 x 1.4 ml tubes on the cooling and heating system of the BioRobot M48 worktable	9015180
QIAshredder (50)	50 disposable cell-lysate homogenizers for use in nucleic acid minipreps, caps	79654
QIAshredder (250)	250 disposable cell-lysate homogenizers for use in nucleic acid minipreps, caps	79656
TissueLyser	Universal laboratory mixermill disruptor	Inquire
TissueLyser Adapter Set 2 x 24	2 sets of Adapter Plates and 2 racks for use with 2.0 ml microcentrifuge tubes on the TissueLyser	69982

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

www.qiagen.com

Australia = Orders 1-800-243-800 = Fax 03-9840-9888 = Technical 1-800-243-066

Austria = Orders 0800-28-10-10 = Fax 0800/28-10-19 = Technical 0800-28-10-11

Belgium = Orders 0800-79612 = Fax 0800-79611 = Technical 0800-79556

Brazil = Orders 0800-557779 = Fax 55-11-5079-4001 = Technical 0800-557779

Canada = Orders 800-572-9613 = Fax 800-713-5951 = Technical 800-DNA-PREP (800-362-7737)

China = Orders 86-21-3865-3865 = Fax 86-21-3865-3965 = Technical 800-988-0325

Denmark = Orders 80-885945 = Fax 80-885944 = Technical 80-885942

Finland = Orders 0800-914416 = Fax 0800-914415 = Technical 0800-914413

France = Orders 01-60-920-926 = Fax 01-60-920-925 = Technical 01-60-920-930 = Offers 01-60-920-928

Germany = Orders 02103-29-12000 = Fax 02103-29-22000 = Technical 02103-29-12400

Hong Kong = Orders 800 933 965 = Fax 800 930 439 = Technical 800 930 425

Ireland = Orders 1800 555 049 = Fax 1800 555 048 = Technical 1800 555 061

Italy = Orders 800-789-544 = Fax 02-334304-826 = Technical 800-787980

Japan = Telephone 03-6890-7300 = Fax 03-5547-0818 = Technical 03-6890-7300

Korea (South) = Orders 080-000-7146 = Fax 02-2626-5703 = Technical 080-000-7145

Luxembourg = Orders 8002-2076 = Fax 8002-2073 = Technical 8002-2067

Mexico = Orders 01-800-7742-639 = Fax 01-800-1122-330 = Technical 01-800-7742-639

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Norway = Orders 800-18859 = Fax 800-18817 = Technical 800-18712

Singapore = Orders 1800-742-4362 = Fax 65-6854-8184 = Technical 1800-742-4368

Spain = Orders 91-630-7050 = Fax 91-630-5145 = Technical 91-630-7050

Sweden = Orders 020-790282 = Fax 020-790582 = Technical 020-798328

Switzerland = Orders 055-254-22-11 = Fax 055-254-22-13 = Technical 055-254-22-12

UK = Orders 01293-422-911 = Fax 01293-422-922 = Technical 01293-422-999

USA = Orders 800-426-8157 = Fax 800-718-2056 = Technical 800-DNA-PREP (800-362-7737)

