June 2011

REPLI-g® Screening Handbook

REPLI-g Screening Kit

For whole genome amplification from purified genomic DNA, blood, and cells

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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- microRNA research and RNAi
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Contents

Kit Contents

REPLI-g Screening Kit	
Catalog no.	**150127**
Number of 40 µl reactions (up to 8 µg yield)	**1000**
REPLI-g Mini DNA Polymerase (blue lid)	1 ml
Buffer SB1 (white lid)	10 x 1.7 ml
Buffer SB2 (yellow lid)	10 x 1.7 ml
Water	3 x 1.8 ml
Handbook	1

Shipping and Storage

The REPLI-g Screening Kit is shipped on dry ice. The kit, including all reagents and buffers, should be stored immediately upon receipt at –20°C in a constant-temperature freezer. When stored under these conditions and handled correctly, the products can be kept at least 6 months after shipping without showing any reduction in performance. For longer storage the kit should be stored at –70°C.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of REPLI-g Screening Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

The REPLI-g Screening Kit is intended for molecular biology applications. This product is 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.

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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. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

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).

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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 REPLI-g Screening 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).

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.

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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

Introduction

The REPLI-g Screening Kit contains DNA polymerase, buffers, and reagents for whole genome amplification from small samples using Multiple Displacement Amplification (MDA) (1). This handbook contains protocols for amplification of DNA from various samples, including purified DNA, whole blood, and tissue culture cells.

Genotyping and DNA sequence analysis of samples can be limited by the small amount of sample available. The REPLI-g Kit allows uniform amplification of whole genomic DNA from small samples, enabling a greater variety and number of analyses to be performed.

Typical DNA yields from a REPLI-g Screening Kit reaction are up to 8 µg per 40 µl reaction. The yield may depend on the quality of the sample. The average product length is typically greater than 10 kb, with a range between 2 kb and 100 kb.

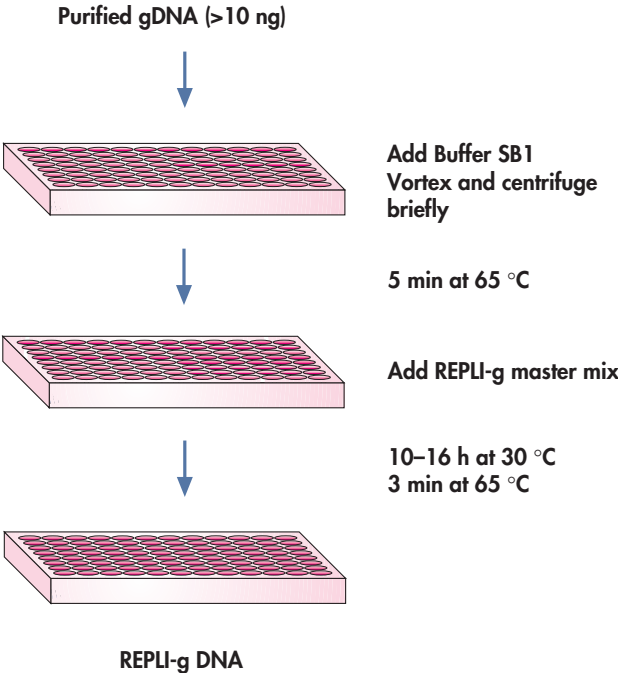
Traditional methods of genomic DNA amplification include the time-consuming process of creating EBV-transformed cell lines followed by whole genome amplification using random or degenerate oligonucleotide-primed PCR. However, all PCR-based methods can generate nonspecific amplification artifacts, give incomplete coverage of loci, and generate DNA less than 1 kb long that cannot be used in many downstream applications. This amplification bias results in an unreliable template for molecular testing.

Principle and procedure

The sample material is lysed and the DNA is denatured by incubating in Buffer SB1 at 65°C. After denaturation has been stopped by cooling the solution down to room temperature, Buffer SB2 and DNA polymerase are added. The isothermal amplification reaction proceeds for at least 10 hours or overnight at 30°C.

REPLI-g Kits provide highly uniform amplification across the entire genome, with negligible sequence bias (2). The method is based on MDA technology, which carries out isothermal genome amplification utilizing a uniquely processive DNA polymerase capable of replicating up to 100 kb without dissociating from the genomic DNA template. The DNA polymerase has a 3'→5' exonuclease proofreading activity to maintain high fidelity during replication and is used in the presence of exonuclease-resistant primers to achieve high yields of DNA product.

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

- Microcentrifuge tubes
- Water bath or heating block
- Microcentrifuge
- Vortexer
- Pipets and pipet tips
- Ice

Protocol: Amplification of Purified Genomic DNA Using the REPLI-g Screening Kit

Important points before starting

- This protocol is optimized for whole genome amplification from >10 ng genomic DNA template. The template DNA should be suspended in TE.* Smaller amounts (1–10 ng) of starting material can be used if the DNA is of sufficient quality.
- For best results, the template DNA should be >2 kb in length with some fragments >10 kb.
- REPLI-g Mini DNA Polymerase should be thawed on ice (see step 4). Buffer SB1 and Buffer SB2 should be thawed at room temperature (15–25°C).

Things to do before starting

- Set a water bath or heating block to 30°C
- Thaw Buffer SB1 and Buffer SB2 at room temperature.
- Buffer SB1 and Buffer SB2 should be vortexed for at least 10 s before use to ensure thorough mixing.

Procedure

1. **Place 2.5–5 µl template DNA into a well of a 96-well plate or a microcentrifuge tube.**

   The amount of template DNA should be >10 ng.

   A DNA control reaction can be set up using 10 ng (1 µl) control genomic DNA (e.g., REPLI-g Human Control Kit, cat. no. 150090). Adjust the volume with TE to the starting volume of your sample.

2. **Add 17 µl Buffer SB1 to the DNA. Mix by vortexing and centrifuge briefly.**

   If using a 96-well plate, seal the plate using a tape sheet before vortexing.

3. **Incubate the sample for 5 min at 65°C. Allow samples to cool down to room temperature (15–25°C).**
4. **Thaw REPLI-g Mini DNA Polymerase on ice.**
5. **Prepare a master mix according to Table 1 on page 10. Mix and centrifuge briefly.**

* 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.

Table 1. Preparation of Master Mix

Component	Volume/reaction	Volume/ 100 reactions
Buffer SB2	17 µl	1700 µl
REPLI-g Mini DNA Polymerase	1 µl	100 µl
Total volume	**18 µl**	**1800 µl**

6. **Add 18 µl of the master mix to 19.5–22 µl of denatured DNA (step 3).**
7. **Incubate at 30°C for 10–16 h.**

   Maximum DNA yield is achieved using an incubation time of 16 h.

   After incubation at 30°C, heat the water bath or heating block up to 65°C if the same water bath or heating block will be used in step 8.
8. **Inactivate REPLI-g Mini DNA Polymerase by heating the sample for 3 min at 65°C.**
9. **Store amplified DNA at 4°C for short-term storage or –20°C for long-term storage.**

   DNA amplified using REPLI-g Kits should be treated as genomic DNA with minimal freeze–thaw cycles. Storage of nucleic acids at low concentration over a long period of time may result in acid hydrolysis. We therefore recommend storage of nucleic acids at a concentration of at least 100 ng/µl.

Protocol: Amplification of Genomic DNA from Blood or Cells Using the REPLI-g Screening Kit

Important points before starting

- The protocol is optimized for 0.5 µl whole blood or cell material (e.g., sorted cells, tissue cultured cells, etc). The cell concentration should be >600 cells/µl. High concentrations of heparin in blood samples can inhibit the REPLI-g reaction (see Troubleshooting Guide, page 14). Blood stabilized in EDTA or citrate may yield better results.
- REPLI-g Mini DNA Polymerase should be thawed on ice (see step 4). All other components can be thawed at room temperature.

Things to do before starting

- Set a water bath or heating block to 30°C.
- Thaw Buffer SB1 and Buffer SB2 at room temperature.
- Buffer SB1 and Buffer SB2 should be vortexed for at least 10 s before use to ensure thorough mixing.

Procedure

1. **Add 0.5 µl cell material (>600 cells/µl) or 0.5 µl blood to each well of a 96-well plate or a microcentrifuge tube.**

   A DNA control reaction can be set up using 10 ng (1 µl) control genomic DNA (e.g., REPLI-g Human Control Kit, cat. no. 150090).

2. **Add 17 µl Buffer SB to each sample. Mix by vortexing and centrifuge briefly.**

   If using a 96-well plate, seal the plate using a tape sheet before vortexing.

3. **Incubate the sample for 5 min at 65°C. Allow samples to cool down to room temperature (15–25°C).**
4. **Thaw REPLI-g Mini DNA Polymerase on ice.**
5. **Prepare a master mix according Table 2 on page 12. Mix and centrifuge briefly.**

* 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.

Table 2. Preparation of Master Mix

Component	Volume/reaction	Volume/ 100 reactions
Buffer SB2	17 µl	1700 µl
REPLI-g Mini DNA-Polymerase	1 µl	100 µl
Total volume	**18 µl**	**1800 µl**

6. **Add 18 µl of the master mix to 17.5 µl of denatured DNA (step 3).**
7. **Incubate at 30°C for 10–16 h.**

   Maximum DNA yield is achieved using an incubation time of 16 h.

   After incubation at 30°C, heat the water bath or heating block up to 65°C if the same water bath or heating block will be used in step 8.
8. **Inactivate REPLI-g Mini DNA Polymerase by heating the sample at 65°C for 3 min.**
9. **Store amplified DNA at 4°C for short-term storage or –20°C for long-term storage.**

   DNA amplified using REPLI-g Kits should be treated as genomic DNA with minimal freeze–thaw cycles. Storage of nucleic acids at low concentration over a long period of time may result in acid hydrolysis. We therefore recommend storage of nucleic acids at a concentration of at least 100 ng/µl.

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).

All protocols

	Comments and suggestions
Reduced or no high-molecular-weight product in agarose gel in some samples, but DNA yield in other samples is approximately 5 µg	
Reaction failed. Possible inhibitor (e.g., salts, heparin etc) in the sample	Clean up or dilute the genomic DNA or blood/cells and re-amplify.
DNA yields of approximately 5 µg in negative (no-template) controls but no positive result in downstream assay (e.g., PCR)	
DNA is generated during REPLI-g reaction by random extension of primer dimers	High-molecular-weight product can be generated by random extension of primer-dimers. This DNA will not affect the quality of actual samples or specific down-stream genetic assays.
DNA yields of approximately 5 µg in negative (no-template) controls and positive result in downstream assay (e.g., PCR)	
DNA is generated during REPLI-g reaction by contaminating DNA templates	Decontaminate all laboratory equipment, and take all necessary precautions to avoid contamination of reagents and samples with extraneous DNA. If possible, work in a laminar-flow hood. Use sterile equipment and barrier pipette tips only, and keep amplification chemistry and DNA templates in separate storage locations.

Genomic DNA protocol

Reduced or no locus representation (allele dropout) in real-time PCR analysis	
Genomic DNA template is degraded	Use intact genomic DNA template. Use larger amount of gDNA.

Blood and cell protocol

Reduced or no locus representation (allele dropout) in real-time PCR analysis

Higher than normal concentration of heparin used as blood	Dilute the heparin-treated blood up to 5-fold using 1x PBS.*

* 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.

Appendix A: Determination of DNA Concentration and Yield

Quantification of DNA yield

A 40 µl REPLI-g reaction typically yields approximately 5 µg of DNA regardless of the amount of template DNA, allowing direct use of the amplified DNA in most downstream genotyping experiments. However, if a more accurate quantification of DNA is required, it is important to utilize a DNA quantification method that is specific for double-stranded DNA, since REPLI-g Kit amplification products contain unused reaction primers. PicoGreen® reagent displays enhanced binding to double-stranded DNA and may be used, in conjunction with a fluorometer, to quantify the double-stranded DNA product. For best results, the sample should be diluted with 2 volumes of water and thoroughly mixed prior to addition of PicoGreen. A protocol for the quantification of REPLI-g amplified DNA can be found in Appendix B.

Quantification of locus representation

Locus representation for each sample can be quantified by real-time PCR (3). Contact QIAGEN Technical Services or visit our Web site at www.qiagen.com for more information.

Appendix B: PicoGreen Quantification of REPLI-g Amplified DNA

This protocol is designed for quantification of double stranded REPLI-g amplified DNA using PicoGreen reagent.

Note: Degraded or old PicoGreen reagent may result in inaccurate DNA quantification. DNA yields in excess of 20 µg should be ignored and, if necessary, quantification should be repeated using fresh PicoGreen reagent.

Important: When working with hazardous chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Equipment and reagents to be supplied by user

- Quant-iT™ PicoGreen dsDNA reagent (Invitrogen, cat. no. P7581)
- TE buffer (10 mM Tris·Cl; 1 mM EDTA, pH 8.0)
- Human genomic DNA (e.g., Promega, cat. no. G3041)
- 2 ml microcentrifuge tube
- 96-well plates (suitable for use in a fluorescence microplate reader)
- Fluorescence microplate reader (e.g., TECAN® Ultra)

Procedure

1. **In a 2 ml microcentrifuge tube, make a 1:150 dilution of PicoGreen stock solution in TE buffer. 20 µl is required for each quantification reaction. Cover the microcentrifuge tube in aluminum foil or place it in the dark to avoid photodegradation of the PicoGreen reagent.**

   For example, to prepare enough PicoGreen working solution for 100 samples, add 13.3 µl PicoGreen to 1986.7 µl TE buffer.

   Important: Prepare the PicoGreen/TE solution in a plastic container as the PicoGreen reagent may adsorb to glass surfaces.

2. **Prepare a 16 µg/ml stock solution of genomic DNA in TE buffer.**
3. **Make 200 µl of 1.6, 0.8, 0.4, 0.2, and 0.1 µg/ml DNA standards by further diluting the 16 µg/ml genomic DNA with TE buffer.**
4. **Transfer 20 µl of each DNA standard in duplicate into a 96-well plate labeled A (see figure below).**

   Note: The 96-well plate must be suitable for use in a fluorescent microplate reader.

96-Well Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H			1.6	0.8	0.4	0.2	0.1	1.6	0.8	0.4	0.2	0.1

Gray squares: genomic DNA standard (ng/µl).

5. **Place 2 µl of each REPLI-g amplified DNA sample for quantification into a new 96-well plate and add 198 µl TE buffer to make a 1/100 dilution. Store the remaining REPLI-g amplified DNA at –20°C.**
6. **Place 2 µl diluted REPLI-g DNA (from step 5) into an unused well of 96-well plate A and add 18 µl TE to make a 1/1000 dilution.**

   The 1/100 dilutions can be stored at –20°C and used for future downstream sample analysis.

7. **Add 20 µl PicoGreen working solution (from step 1) to each sample (amplified DNA and DNA standards) in 96-well plate A. Gently shake the plate on the bench top to mix the samples and reagent.**
8. **Centrifuge the 96-well plate briefly to collect residual liquid from the walls of the wells.**
9. **Measure the sample fluorescence using a fluorescence microplate reader and standard fluorescence filters (excitation approximately 480 nm; emission approximately 520 nm).**

   To ensure that the sample readings remain in the detection range of the microplate reader, adjust the instrument's gain so that the sample with the highest DNA concentration yields fluorescence intensity near the fluorimeter's maximum.

Calculation of DNA concentration and yield

10. **Generate a standard curve by plotting the concentration of DNA standards (µg/ml) (X-axis) against the fluorescence reading generated by the microplate reader (Y-axis). Plot an average of the fluorescence recorded for each DNA standard of the same concentration.**
11. **Use the standard curve to determine the concentration (µg/ml) of the diluted REPLI-g amplified DNA sample. This is achieved by plotting the fluorescence reading of the sample against the standard curve and reading the DNA concentration on the X-axis.**

    Note: The calculation of DNA concentration depends on the standard curve and the determination of the slope. For accurate results, the standard curve should be a straight line. Any deviation from this may cause inaccuracies in the measurement of REPLI-g amplified DNA concentrations.
12. **Multiply the value determined in step 11 by 1000 to show the concentration of undiluted sample DNA (as the sample DNA measured by PicoGreen fluorescence had been diluted 1 in 1000).**
13. **To determine the total amount of DNA in your sample, multiply the concentration of undiluted sample DNA (µg/ml) (step 12) by the reaction volume in milliliters (i.e., for a 40 µl reaction, multiply by 0.04).**

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Cited references

1. Dean, F. B. et al. (2002) Comprehensive human genome amplification using multiple displacement amplification. Proc. Natl. Acad. Sci. USA **99**, 5261.
2. Hosono, S. et al. (2003) Unbiased whole-genome amplification directly from clinical samples. Genome Res. **13**, 954.
3. Yan, J. et al. (2004) Assessment of multiple displacement amplification in molecular epidemiology. Biotechniques **37**, 136.

Ordering Information

Product	Contents	Cat. no.
REPLI-g Screening Kit — for high-throughput manual or automated whole genome amplification from small or precious samples		
REPLI-g Screening Kit (1000)	For 1000 whole genome amplification reactions: REPLI-g Mini DNA Polymerase, Buffers	150127
REPLI-g Mini and Midi Kits — for highly uniform whole genome amplification from small or precious samples		
REPLI-g Mini Kit (25)	For 25 whole genome amplification reactions: REPLI-g Mini DNA Polymerase, Buffers	150023
REPLI-g Mini Kit (100)	For 100 whole genome amplification reactions: REPLI-g Mini DNA Polymerase, Buffers	150025
REPLI-g Midi Kit (25)	For 25 whole genome amplification reactions: REPLI-g Midi DNA Polymerase, Buffers	150043
REPLI-g Midi Kit (100)	For 100 whole genome amplification reactions: REPLI-g Midi DNA Polymerase, Buffers	150045
REPLI-g Service — large-scale highly uniform whole genome amplification and quality assessment from limited or precious samples		
REPLI-g Service, Single Tube (100 µg)	Whole Genome Amplification Service from single tubes or <84 samples in microplates, 100 µg scale	805923
REPLI-g Service, Single Tube (500 µg)	Whole Genome Amplification Service from single tubes or <84 samples in microplates, 500 µg scale	805925
REPLI-g Service (100 µg)	Whole Genome Amplification Service from microplates, 100 µg scale	805943
REPLI-g Service (500 µg)	Whole Genome Amplification Service from microplates, 500 µg scale	805945

Ordering Information

Product	Contents	Cat. no.
Related products		
REPLI-g Human Control Kit (25)	Human control DNA for 25 x 50 µl whole genome amplification reactions	150090
QIAamp® DNA Blood Mini Kit (50)*	For 50 DNA minipreps: 50 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml)	51104
QIAamp DNA Micro Kit (50)	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Carrier RNA, Buffers, Collection Tubes (2 ml)	56304
QIAamp DNA Mini Kit (50)*	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304
QuantiTect® Probe PCR Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Probe PCR Master Mix, 2 x 2.0 ml RNase-free water	204343
QuantiTect Multiplex PCR Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Multiplex PCR Master Mix (contains ROX™ dye), 2 x 2 ml RNase-Free Water	204543
QIAGEN Multiplex PCR Kit (100)*	For 100 x 50 µl multiplex PCR reactions: 2x QIAGEN Multiplex PCR Master Mix (providing a final concentration of 3 mM $MgCl_2$, 3 x 0.85 ml), 5x Q-Solution® (1 x 2.0 ml), distilled water (2 x 1.7 ml)	206143

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* Larger kit sizes available; please inquire.

Notes

Notes

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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

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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

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