FastLane Kits — from Sample Direct to Result

New

QIAGEN®

Sample & Assay Technologies

Speed up and simplify your workflow

FastLane Kits accelerate and streamline real-time RT-PCR analysis of cultured cells. By eliminating the need for RNA purification, the kits allow you to carry out real-time RT-PCR directly from cell lysates. The kits are ideal for experiments requiring rapid, high-throughput gene expression analysis, such as validation of siRNA-mediated gene knockdown.

Benefits of FastLane Kits:

- No RNA purification, significantly saving time
- Just 3 steps from cells to real-time RT-PCR
- High-throughput analysis of even 384-well plates
- RNA detection only due to unique gDNA Wipeout Buffer
- Immediate startup using optimized reagents and protocols

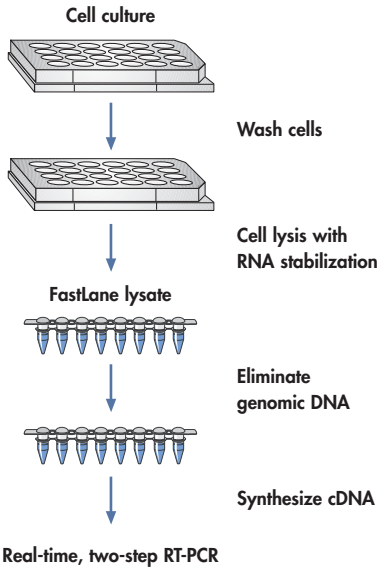
Table 1. FastLane Kits for Real-Time RT-PCR

Procedure	Detection method	Cell-culture formats	Kit	Page
Two-step RT-PCR	All	Up to 96-well*	FastLane Cell cDNA Kit	3
One-step RT-PCR	SYBR® Green	Up to 384-well†	FastLane Cell SYBR Green Kit	4
One-step RT-PCR	Probe	Up to 384-well†	FastLane Cell Probe Kit	5
One-step RT-PCR	Multiplex	Up to 384-well†	FastLane Cell Multiplex Kits	6

* 96-well format requires addtionally one QuantiTect® Reverse Transcription Kit (50).

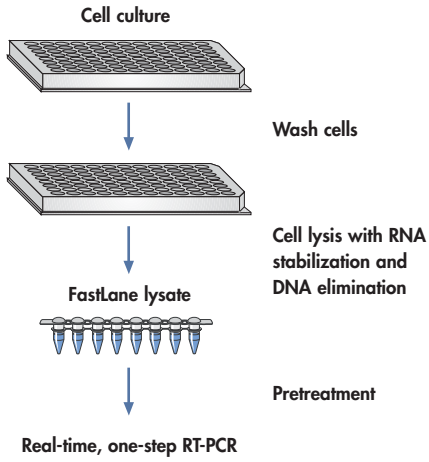
† 384-well format requires either a reduction of PCR volume from 50 µl to at least 25 µl, or an additional QuantiTect RT-PCR Kit; please inquire.

FastLane Two-Step RT-PCR Procedure



FastLane lysate in 12 minutes.
cDNA (PCR template) in 45 minutes.

FastLane One-Step RT-PCR Procedure



FastLane lysate (PCR template)
in 12 minutes.

High-speed procedure for cDNA from cells

Benefits of the FastLane Cell cDNA Kit:

- Only 4 steps from cells to cDNA
- Sensitive RT-PCR due to high cDNA yields
- High reproducibility in cDNA preparation
- Quick snapshot of gene expression profiles

cDNA synthesized by the FastLane Cell cDNA Kit enables highly sensitive and reproducible results in real-time, two-step RT-PCR (Figures 1 and 2). The cDNA also allows accurate detection over a wide linear range from >10^5 cells down to one cell.

To ensure only RNA is detected in real-time RT-PCR, novel gDNA Wipeout Buffer is used to eliminate genomic DNA. Time and effort are saved as there is no need to design RNA-specific primers or probes.

When the FastLane Cell cDNA Kit is used together with optimized QuantiTect Kits for real-time RT-PCR, several cell samples can be easily processed and analyzed within a few hours. This allows a snapshot of several transcripts in experiments such as validation of siRNA-mediated gene knockdown (Figure 3).

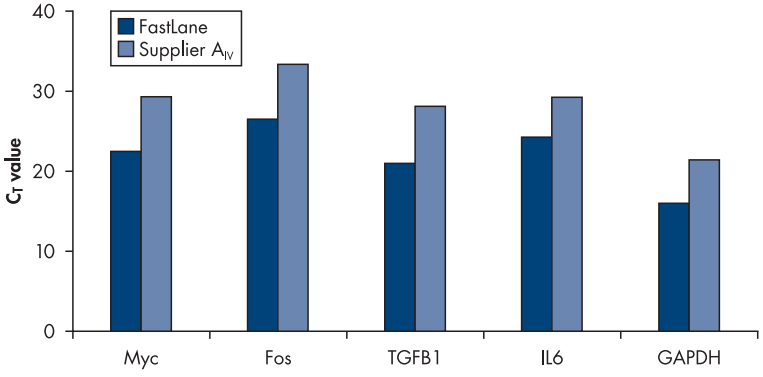


Figure 1. Superior sensitivity in analysis of cancer genes. In real-time, two-step RT-PCR analysis of various cancer genes in HeLa cells, lower C_T values were achieved when cDNA was prepared with the FastLane Kit instead of a similar kit from Supplier A_{IV}.

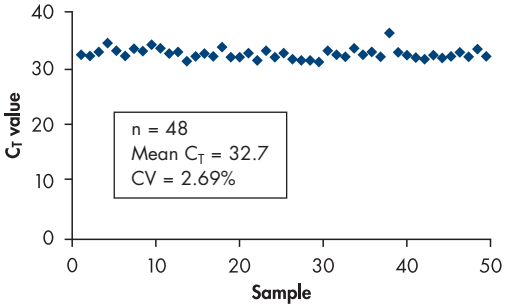


Figure 2. Highly reproducible cDNA preparation. cDNA was prepared from a 48-well plate containing HeLa cells using the FastLane Kit. Expression of tubulin B (structural protein) was analyzed by real-time, two-step RT-PCR.

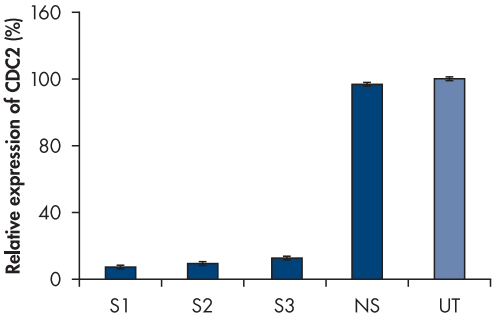


Figure 3. Successful analysis of CDC2 knockdown. MCF-7 cells were transfected with various CDC2 siRNAs (**S1**, **S2**, or **S3**) or nonsilencing siRNA (**NS**). After 48 hours, cDNA was prepared using the FastLane Kit. Expression of CDC2 (cell cycle protein) relative to GAPDH (endogenous control) was analyzed by real-time, two-step RT-PCR. The relative expression of CDC2 in untreated cells (**UT**) was set to 100%.

See page 2 for more FastLane benefits!

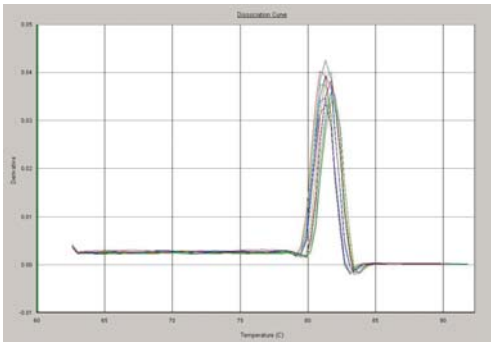


Figure 4. Highly specific quantification. Real-time, one-step RT-PCR using the FastLane Kit and a QuantiTect Primer Assay for INPP5D (a phosphatase) enabled specific transcript quantification in HeLa, HepG2, and Cos cells, as shown by the single peaks in melting curve analysis.

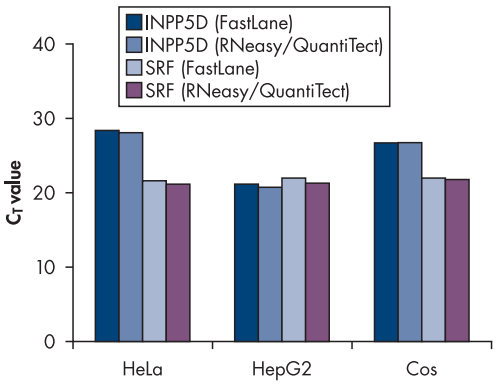


Figure 5. Sensitive detection without RNA purification. Two low-abundance transcripts, INPP5D (a phosphatase) and SRF (serum response factor), were quantified by real-time, one-step RT-PCR. The FastLane Kit provided C_T values comparable to those achieved with the RNeasy® Mini Kit and QuantiTect SYBR Green RT-PCR Kit.

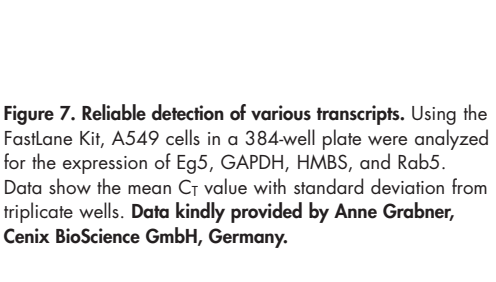


Figure 7. Reliable detection of various transcripts. Using the FastLane Kit, A549 cells in a 384-well plate were analyzed for the expression of Eg5, GAPDH, HMBS, and Rab5. Data show the mean C_T value with standard deviation from triplicate wells. **Data kindly provided by Anne Grabner, Cenix BioScience GmbH, Germany.**

High-speed, specific SYBR Green detection

Benefits of the FastLane Cell SYBR Green Kit:

- High specificity in SYBR Green quantification
- Sensitive detection of low-abundance transcripts
- Reproducible results in high-throughput analysis
- Easy processing of 96- or 384-well plates

The FastLane Cell SYBR Green Kit uses a unique RT-PCR buffer to prevent the formation of nonspecific products and primer–dimers, allowing highly specific quantification in real-time, one-step RT-PCR (Figure 4). Sensitive quantification is also achieved despite the absence of an RNA purification step (Figure 5). Results in high-throughput experiments are very reproducible (Figures 6 and 7) and not affected by genomic DNA contamination.

Specific transcript detection is guaranteed when the kit is used in combination with QuantiTect Primer Assays. These are predesigned, genomewide primer sets, available at www.qiagen.com/GeneGlobe .

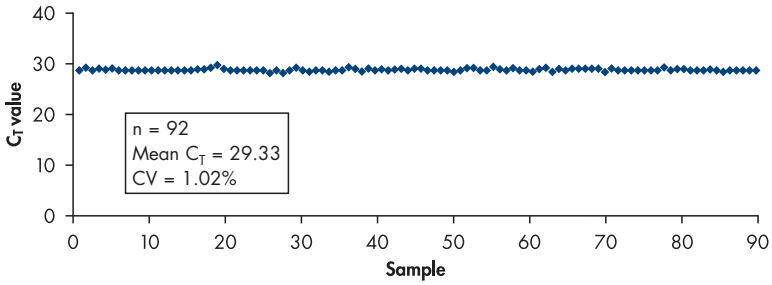


Figure 6. Highly reproducible analysis of a 96-well plate. Real-time, one-step RT-PCR using the FastLane Kit and a QuantiTect Primer Assay for INPP5D (a phosphatase) gave reproducible C_T values from a 96-well plate of HeLa ACC cells.

See page 2 for more FastLane benefits!

High-speed, reproducible probe quantification

Benefits of the FastLane Cell Probe Kit:

- Proven probe detection without RNA purification
- Sensitive probe detection of low-copy targets
- Reproducible results in high-throughput analysis
- Easy processing of 96- or 384-well plates

The FastLane Cell Probe Kit delivers sensitive probe-based detection in real-time, one-step RT-PCR without the need for RNA purification (Figure 8). Real-time quantification is more sensitive and linear compared with similar kits (Figures 9 and 10), and is highly reproducible (Figure 11). In addition, genomic DNA does not interfere with quantification.

All types of probe chemistry are compatible with the FastLane Cell Probe Kit. These include TaqMan® or other dual-labeled probes, LightCycler® hybridization (FRET) probes, and Molecular Beacons. A ready-to-use RT-PCR master mix eliminates the need to optimize probe concentration and other PCR parameters.

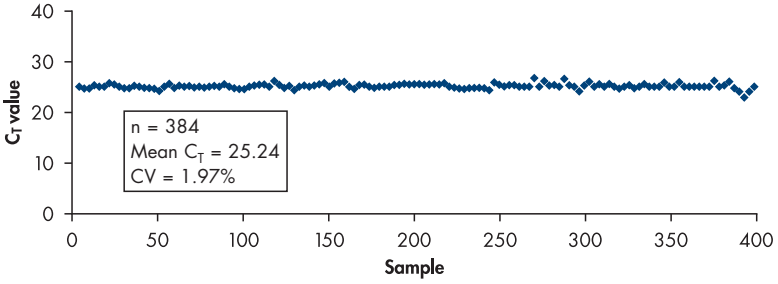


Figure 11. Highly reproducible analysis of a 384-well plate. Real-time, one-step RT-PCR analysis of the expression of NFκB1 (nuclear transcription factor) using the FastLane Kit gave reproducible C_T values from a 384-well plate of HeLa cells.

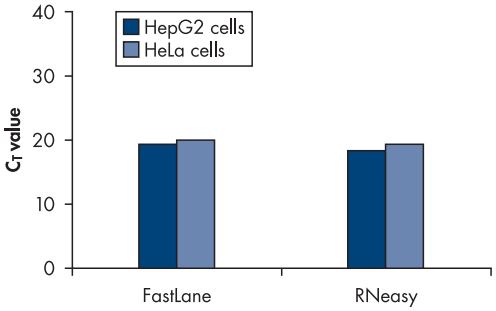


Figure 8. Sensitive probe detection without RNA purification. Expression of β-actin (cytoskeleton protein) was analyzed by real-time, one-step RT-PCR. The FastLane Kit provided C_T values comparable to those achieved with the RNeasy Mini Kit and QuantiTect Probe RT-PCR Kit.

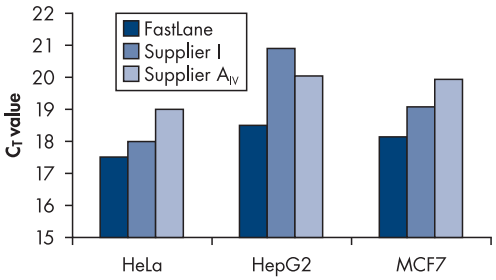


Figure 9. Greater sensitivity than other kits. The FastLane Kit gave lower C_T values in real-time, one-step RT-PCR than similar kits from other suppliers. Each kit was used with an assay for PPIA (an isomerase).

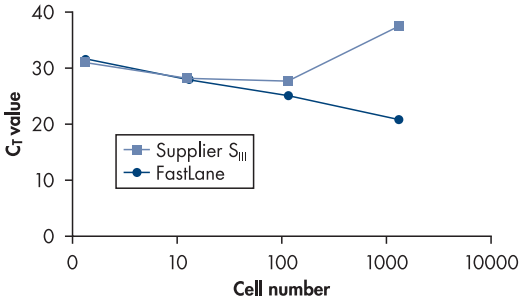


Figure 10. Linear detection down to a single cell. The FastLane Kit enabled linear detection of β-actin transcript in 1–10,000 HeLa cells (serial dilutions of lysate were analyzed). In contrast, a similar kit from Supplier S_{III} showed no linearity.

See page 2 for more FastLane benefits!

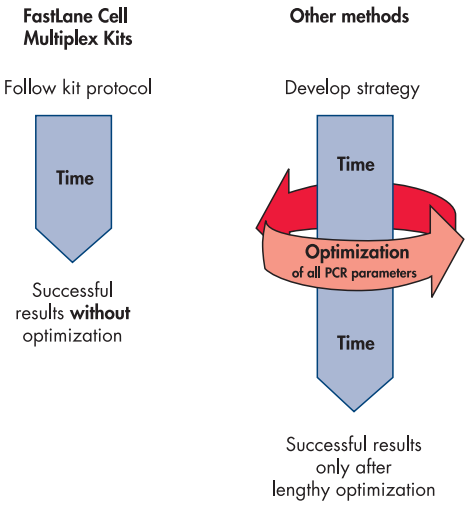


Figure 12. Multiplex analysis with no optimization steps. FastLane Cell Multiplex Kits avoid continuous rounds of optimization of PCR parameters.

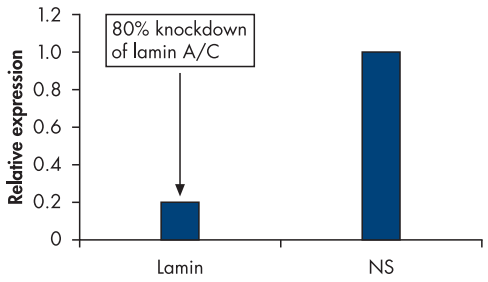


Figure 13. Reliable validation of gene silencing. In a 96-well plate of HCT116 cells, 5 wells were transfected with lamin A/C siRNA (**Lamin**) or nonsilencing siRNA (**NS**). Cells were analyzed by multiplex, real-time RT-PCR using a FastLane Kit and TaqMan assays for lamin A/C (structural protein) and 18S rRNA (endogenous control). **Data kindly provided by Angela Quinn, Genzyme Corporation, USA.**

High-speed analysis of up to 5 targets

Benefits of FastLane Cell Multiplex Kits:

- First multiplex RT-PCR kits to forgo RNA purification
- No optimization of reaction conditions required
- Reproducible results in high-throughput analysis
- Up to 5 targets analyzed per well

Unlike other methods, FastLane Cell Multiplex Kits deliver success in multiplex, real-time, one-step RT-PCR at the first attempt (Figure 12). This is due to an optimized master mix containing a novel RT-PCR buffer and a hot-start DNA polymerase. Multiplex analysis in 96- and 384-well formats is highly reproducible (Figure 14).

The kits are ideal for rapid, high-throughput gene expression analysis, such as validation of siRNA-mediated gene knockdown (Figure 13). Target and control genes are amplified in the same reaction with similar, high PCR efficiencies, enabling relative quantification by the $\Delta\Delta C_T$ method.

Two kit formats are available: the FastLane Cell Multiplex Kit for cyclers that require ROX dye for fluorescence normalization, and the FastLane Cell Multiplex NR Kit for all other cyclers.

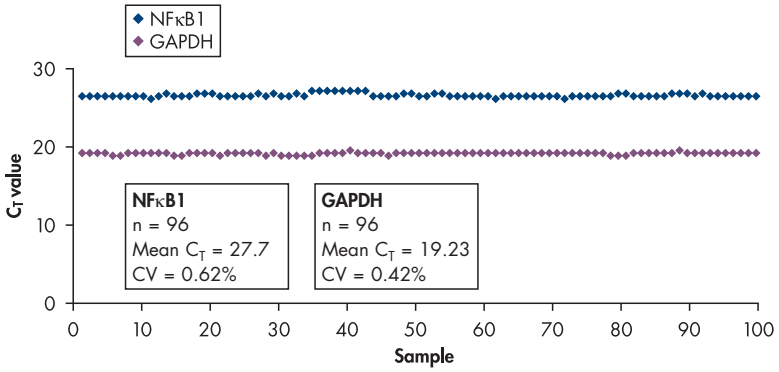


Figure 14. Highly reproducible analysis of a 96-well plate. Multiplex, real-time, one-step RT-PCR analysis of the expression of NFκB1 (nuclear transcription factor) and GAPDH (endogenous control) using the FastLane Kit gave reproducible C_T values from a 96-well plate of HeLa cells.

See page 2 for more FastLane benefits!

Ordering Information

Product	Contents	Cat. No.
FastLane Cell cDNA Kit (50)	Buffer FCW, Buffer FCP, and components for 50 x 20 µl reverse-transcription reactions	215011
FastLane Cell SYBR Green Kit (200)	FastLane Cell One-Step Buffer Set and FastLane Cell SYBR Green RT-PCR Mixes	216213
FastLane Cell Probe Kit (200)	FastLane Cell One-Step Buffer Set and FastLane Cell Probe RT-PCR Mixes	216413
FastLane Cell Multiplex Kit (200)*	FastLane Cell One-Step Buffer Set and FastLane Cell Multiplex RT-PCR Mixes	216513
FastLane Cell Multiplex NR Kit (200)†	FastLane Cell One-Step Buffer Set and FastLane Cell Multiplex RT-PCR NR Mixes	216713
Products for two-step RT-PCR (SYBR Green, probe, or multiplex detection)		
QuantiTect SYBR Green PCR Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water	204143
QuantiTect Probe PCR Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water	204343
QuantiTect Multiplex PCR Kit (200)*‡	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix (with ROX dye), 2 x 2 ml RNase-Free Water	204543
QuantiTect Reverse Transcription Kit (50)	Components for 50 x 20 µl reverse-transcription reactions	205311

* Recommended for cyclers from Applied Biosystems.

† Recommended for cyclers from other suppliers. ‡ Kit without ROX dye available for other cyclers; please inquire.

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.

Trademarks: QIAGEN®, QuantiTect®, RNeasy® (QIAGEN Group); LightCycler® (Roche Group); SYBR® (Molecular Probes, Inc.); TaqMan® (Roche Group).

Purchase of the FastLane Cell SYBR Green Kit, QuantiTect SYBR Green PCR Kit, and QuantiTect SYBR Green RT-PCR Kit is accompanied by a limited, non-transferable immunity from suit to use it with detection by a dsDNA-binding dye as described in U.S. Patents Nos. 5,994,056 and 6,171,785 and corresponding patent claims outside the United States for the purchaser's own internal research. No real-time apparatus or system patent rights or any other patent rights, and no right to use this product for any other purpose are conveyed expressly, by implication or by estoppel.

The FastLane Cell Probe Kit, FastLane Cell Multiplex Kits, QuantiTect Probe PCR Kit, QuantiTect Probe RT-PCR Kit, QuantiTect Multiplex PCR Kits, and QuantiTect Multiplex RT-PCR Kits are an Authorized 5' Nuclease Core Kit without Licensed Probe. Its purchase price includes a limited, non-transferable immunity from suit under certain patents owned by Roche Molecular Systems, Inc. or F. Hoffmann-La Roche Ltd, for using only this amount of the product in the practice of the 5' nuclease process solely for the purchaser's own internal research when used in conjunction with Licensed Probe. No right under any other patent claims (such as apparatus or system claims) and no right to use this product for any other purpose is hereby granted expressly, by implication or by estoppel. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Purchase of the FastLane Cell SYBR Green Kit, FastLane Cell Probe Kit, FastLane Cell Multiplex Kits, QuantiTect SYBR Green RT-PCR Kit, QuantiTect Probe RT-PCR Kit, and QuantiTect Multiplex RT-PCR Kits is accompanied by a limited, non-transferable license under RT and Reverse Transcription-PCR patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd to use it for the purchaser's own internal research. No real-time patent rights of any kind, no right under any other patent claims (such as apparatus or system claims), and no right to use this product for any other purpose is hereby granted expressly, by implication or by estoppel.

QuantiTect Primer Assays are compatible for use in the 5' nuclease process or the dsDNA-binding dye processes covered by patents owned by Roche or owned by or licensed to Applera Corporation. No license under these patents to practice the 5' nuclease process or the dsDNA-binding dye processes are conveyed expressly or by implication to the purchaser by the purchase of this product.

© 2010 QIAGEN, all rights reserved.

www.qiagen.com

Australia ▪ Orders 03-9840-9800 ▪ 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

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

China ▪ Orders 021-51345678 ▪ Fax 021-51342500 ▪ Technical 021-51345678

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

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

France ▪ Orders 01-60-920-920 ▪ Fax 01-60-920-925 ▪ Technical 01-60-920-930

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

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

Italy ▪ Orders 02-33430411 ▪ Fax 02-33430426 ▪ Technical 800 787980

Japan ▪ Telephone 03-5547-0811 ▪ Fax 03-5547-0818 ▪ Technical 03-5547-0811

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

The Netherlands ▪ Orders 0800-0229592 ▪ Fax 0800-0229593 ▪ Technical 0800-0229602

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

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)

1063765 07/2010

Sample & Assay Technologies