TaqMan® Exogenous Internal Positive Control Reagents

VIC™ Probe

Protocol

Applied Biosystems

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Contents

Introduction 1
- Purpose of the Kit 1
- Simultaneously Amplifying Two DNAs 1
- Interpreting Negative Results 2
- Custom Applications 2
- End Point Detection and Post-PCR Plate Read 3
- Sequence Detection 3

Materials and Equipment 4
- Kit Contents 4
- Core Kits Supplied by the User 4
- Materials Supplied by User 5
- Storage and Stability 6

Preparing Reactions and Thermal Cycling 7
- Introduction 7
- About Preparing Reactions 7
- Thermal Cycling 8

Performing End Point Detection on the ABI PRISM 7200 or 7700 9
- Overview 9
- Setting up the Software 9

Setting Up the Plates 10
- Overview 10
- FAM Layer 10
- VIC Layer 11

Analyzing Data for End Point Runs 13
- Analyzing Data 13
- Target Template Calls 14

Technical Support 15
Contacting Technical Support 15
To Contact Technical Support by E-Mail 15
Hours for Telephone Technical Support 15
To Contact Technical Support by Telephone or Fax 16
To Reach Technical Support Through the Internet 19
To Obtain Documents on Demand 20
Appendix A. Preventing Contamination 21
Introduction 21
AmpErase UNG 21
General PCR Practices 22
Appendix B. References 23

Introduction

Purpose of the Kit

The TaqMan® Exogenous Internal Positive Control Reagents is a pre-optimized internal positive control (IPC) which can be spiked into samples to distinguish true target negatives from PCR inhibition.

The kit is designed to:

- Distinguish types of negative results

  A negative call for the target sequence and positive call for the IPC indicates that no target sequence is present

  A negative call for the target sequence and negative call for the IPC suggests PCR inhibition

- Avoid amplification of endogenous genes
- Permit coamplifcation of the IPC and the target sequence without compromising amplification of the target sequence
- Perform optimally with the TaqMan® Universal PCR Master Mix

IMPORTANT To obtain +/– assignments with a 99.7% confidence level, a post-PCR plate read should always be performed. Real time (+/–) document assignments on the ABI PRISM 7700 Sequence Detector have not been verified to be statistically significant.

Simultaneously Amplifying Two DNAs

By using the TaqMan Exogenous Internal Positive Control Reagents, a low-copy target DNA can be amplified in the same tube with the IPC. Although the target and IPC DNAs may differ in initial copy number, the amplification efficiency of the target reaction is not compromised. This is achieved by limiting the concentration of IPC primers in the PCR reaction.

In the PCR reaction the IPC is detected using a VIC-labeled probe and the target template is detected using a FAM-labeled probe.

Interpreting Negative Results

The TaqMan Exogenous Internal Positive Control Reagents, in conjunction with your target system, identify samples that are positive and negative for a specific target sequence. The kit distinguishes between two types of negative reactions:

- Samples identified as negative because they lack the target sequence
- Samples identified as negative because of the presence of a PCR inhibitor

During amplification, the sample and IPC generate reporter fluorescence signals such that identification calls may be made on unknown samples.

Positive and negative calls are made on the basis of statistical analysis of data from the two dye layers. The statistical analysis sets up threshold values for positive FAM and VIC calls on the basis of the No Template Control (NTC) and the Negative Internal Positive Control (IPC–) baselines.

In this kit, the FAM layer shows the positive (+) and negative (–) calls for the target template and the VIC layer shows the +/– calls for the IPC. The target template calls are made on the following basis:

If the Detectable Target Template (FAM) call is...	And the Detectable IPC (VIC) call is...	Then the Target Template is...
+	+, –[a]	+
–	+	–
–	–	No Amp / ?

a. In the presence of a strong FAM signal for the target assay, a negative assignment and/or signal can be obtained in the VIC layer. This is a result of the limiting primer concentrations used in the IPC assay.

Custom Applications

The IPC DNA, primers, and probes supplied with this kit can be used with all sample target systems. Refer to the *TaqMan Universal PCR Master Mix Protocol* (P/N 4304449) for instructions on how to optimize your target system's performance.

End Point Detection and Post-PCR Plate Read

The TaqMan Exogenous Internal Positive Control Reagents are designed for Plate Read (end point) detection only. Plate Read detection collects one fluorescent scan per tube after PCR is completed.

The TaqMan Exogenous Internal Positive Control Reagents are designed to utilize the post-PCR plate read function. Utilization of the pre-PCR plate read may interfere with the ability of the system to make accurate +/– assignments for any specific target.

Plate read detection is performed using the following instruments:

- ABI PRISM® 7700 Sequence Detection System
- ABI PRISM® 7200 Sequence Detection System

Sequence Detection

The Sequence Detection Systems from Applied Biosystems are used to measure the increase of reporter fluorescence following PCR. Reporter signals are normalized to the emission of a passive reference:

$$R_{n\,(TT)} = \frac{\text{Emission Intensity of Target Template Sequence}}{\text{Emission Intensity of Passive Reference}}$$

$$R_{n\,(IPC)} = \frac{\text{Emission Intensity of Internal Positive Control}}{\text{Emission Intensity of Passive Reference}}$$

Materials and Equipment

Kit Contents

The TaqMan Exogenous Internal Positive Control Reagents (P/N 4308323) provide sufficient material to perform two hundred 50-µL reactions. There is enough 10X Block for twenty-four 50-µL reactions.

The kit contents are listed in the table below.

Component	Volume	Description
10X Exo IPC Mix	1.0 mL	One tube containing IPC primers and probe.
10X Exo IPC Block	120 µL	One tube containing enough blocking reagent for twenty-four 50-µL reactions.
50X Exo IPC DNA	200 µL	One tube of IPC template DNA.

IMPORTANT The TaqMan VIC dye must be configured as a Pure Dye on the ABI PRISM® 7700/7200 Sequence Detection Systems for it to appear on the Reporter pull-down menu. See *User Bulletin #4: Generating New Spectra Components* (P/N 4306234) pages 6-7 to configure TaqMan VIC as a Pure Dye.

Core Kits Supplied by the User

One of the TaqMan core reagent kits listed in the following table is required in addition to the reagents supplied in the TaqMan Exogenous Internal Positive Control Reagents. The Exogenous IPC Reagents have been optimized with the TaqMan Universal PCR Master Mix.

Application	TaqMan Core Reagents	Source
PCR	TaqMan Universal PCR Master Mix	Applied Biosystems (P/N 4304437)
	TaqMan® PCR Core Reagents	Applied Biosystems (P/N N808-0228)

Materials Supplied by User

The items listed in the following tables are required in addition to the reagents supplied.

Equipment Item	Source
ABI PRISM 7700 Sequence Detection System	See your local Applied Biosystems' representative for the instrument best suited to meet your needs.
ABI PRISM 7200 Sequence Detection System/GeneAmp® PCR System 9600	

Product	Source
Custom TaqMan Probes 5,000 pmol 15,000–25,000 pmol 50,000–100,000 pmol	Applied Biosystems (P/N 450025) (P/N 450024) (P/N 450003)
MicroAmp® Optical 96-Well Reaction Plate and Optical Caps	Applied Biosystems (P/N 403012)
MicroAmp Optical 96-Well Reaction Plate	Applied Biosystems (P/N N801-0560)
MicroAmp Optical Tubes	Applied Biosystems (P/N N801-0933)
MicroAmp Optical Caps	Applied Biosystems (P/N N801-0935)
Deionized water or TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)	Major laboratory suppliers (MLS)

Note The ABI PRISM 7700 and ABI PRISM 7200 Sequence Detectors use the MicroAmp Optical 96-Well Reaction Plate and MicroAmp Optical Caps.

IMPORTANT Do not use MicroAmp Optical Tubes with the ABI PRISM 7200 Sequence Detector.

Storage and Stability

Store the TaqMan Exogenous Internal Positive Control Reagents at –20 to –25 °C. However, if the reagents will be consumed within one month, store them at 2–4 °C. If stored under the recommended conditions, the product will maintain performance for one year from time of receipt.

Preparing Reactions and Thermal Cycling

Introduction

The TaqMan Exogenous IPC Reagents are designed for use with end point detection only.

IMPORTANT To obtain +/– assignments with a 99.7% confidence level, a post-PCR plate read should always be performed. Real time (+/–) document assignments on the ABI PRISM 7700 Sequence Detector have not been verified to be statistically significant.

About Preparing Reactions

Prepare the reactions as described below. Follow precautions to prevent PCR contamination as described in Appendix A on page 21.

Step 1 — Make the following Master Mix and pipet 45 µL into each well of the 96-Well Reaction Plate.

Item	Volume for one Reaction	Volume for 100 Reactions
TaqMan Universal PCR Master Mix	25 µL	2.5 mL
10X Exo IPC Mix	5 µL	0.5 mL
50X Exo IPC DNA	1 µL	0.1 mL
Target primers, probe, and deionized water	14 µL	1.4 mL
Total	45 µL	4.5 mL

Step 2 — Pipet 5 µL of sample into each well of a 96-well plate as shown in "FAM Layer" on page 10.

Note The final reaction volume in each well should be 50 µL.

Well	IF preparing ...	Then add...
A1–A6	NAC	5 µL of 10X Exo IPC Block
A7–A12	NTC	5 µL of 1X TE
B1–H12	UNKN	5 µL of sample

Thermal Cycling

Use the following procedure to amplify samples.

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Place the MicroAmp Optical 96-Well Reaction Plate in the thermal cycler.</td></tr>
<tr><td>2</td><td>Program the thermal cycler:
<table>
<tr><td rowspan="2">Thermal Cycler</td><td colspan="4">Times and Temperatures</td></tr>
<tr><td colspan="2">Initial Steps</td><td colspan="2">Each of 40 Cycles</td></tr>
<tr><td></td><td></td><td>Melt</td><td>Anneal/ Extend</td></tr>
<tr><td rowspan="2">GeneAmp PCR System 9600 or 9700[a]</td><td>HOLD</td><td>HOLD</td><td colspan="2">CYCLE</td></tr>
<tr><td>2 min.
50 °C</td><td>10 min.
95 °C</td><td>15 sec.
95 °C</td><td>1 min.
60 °C</td></tr>
<tr><td rowspan="2">ABI PRISM 7700 Sequence Detector</td><td>HOLD</td><td>HOLD</td><td colspan="2">CYCLE</td></tr>
<tr><td>2 min.
50 °C</td><td>10 min.
95 °C</td><td>15 sec.
95 °C</td><td>1 min.
60 °C</td></tr>
</table>
a. If the 9700 thermal cycler is used, use the 9600 emulation mode.</td></tr>
<tr><td>3</td><td>Perform PCR amplification.</td></tr>
<tr><td>4</td><td>Store the PCR products at 2–6 °C until you are ready for analysis.</td></tr>
</table>

Performing End Point Detection on the ABI PRISM 7200 or 7700

Overview

To perform end point analysis on the ABI PRISM 7200 or 7700 Sequence Detectors follow the procedure described below.

Setting up the Software

To set up the Sequence Detection System software:

Step	Action
1	Open the ABI PRISM Sequence Detection System (SDS) software.
2	Double-click on the File/New Plate. Select single-reporter, plate read, and the correct instrument (7700 or 7200).
3	Define the FAM layer as shown in “FAM Layer” on page 10. See your instrument user’s manual for more information.
4	Define the VIC layer as shown in “VIC Layer” on page 11. See your instrument user’s manual for more information.
5	Click the Show Analysis button.
6	Click the Post PCR Read button. The software will perform the Plate Read. **Note** The TaqMan Exogenous Internal Positive Control Reagents are designed to utilize the post-PCR plate read function. Utilization of the pre-PCR plate read may interfere with the ability of the system to make accurate +/– assignments for any specific target.
7	Save the plate.
8	Proceed to “Analyzing Data for End Point Runs” on page 13.

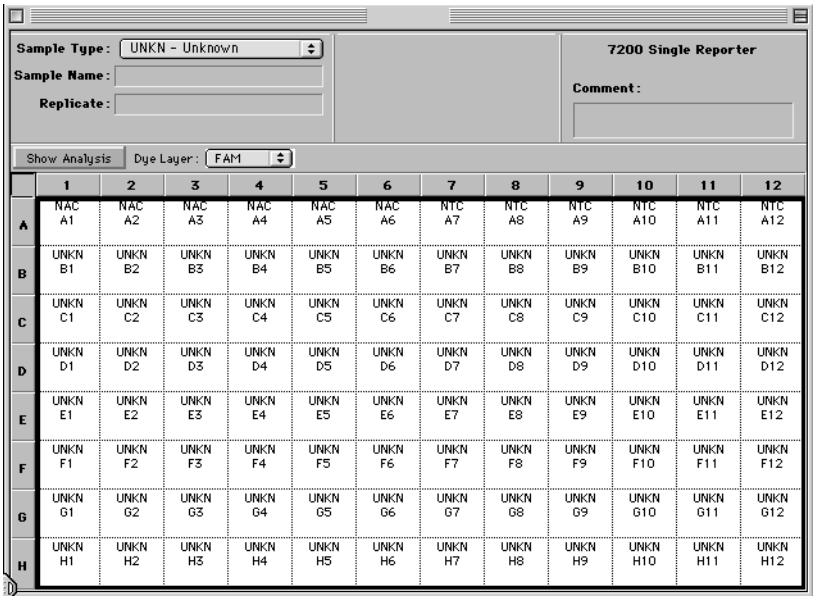
Setting Up the Plates

Overview The plate setup for the FAM layer and the VIC layer are shown.

FAM Layer The FAM layer consists of the following (see figure below):

- Six No Amplification Control (NAC) wells
- Six No Target Template Control (NTC) wells
- Eighty four Unknown (UNKN) wells

IMPORTANT Six replicates of No Template Control must be run to make +/- calls at a 99.7% confidence level. These are required to accurately define the +/- thresholds for the FAM and VIC layers.

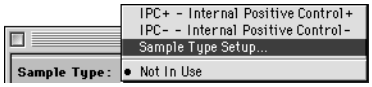
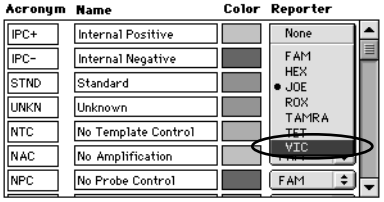
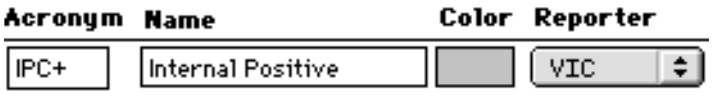


	1	2	3	4	5	6	7	8	9	10	11	12
A	NAC A1	NAC A2	NAC A3	NAC A4	NAC A5	NAC A6	NTC A7	NTC A8	NTC A9	NTC A10	NTC A11	NTC A12
B	UNKN B1	UNKN B2	UNKN B3	UNKN B4	UNKN B5	UNKN B6	UNKN B7	UNKN B8	UNKN B9	UNKN B10	UNKN B11	UNKN B12
C	UNKN C1	UNKN C2	UNKN C3	UNKN C4	UNKN C5	UNKN C6	UNKN C7	UNKN C8	UNKN C9	UNKN C10	UNKN C11	UNKN C12
D	UNKN D1	UNKN D2	UNKN D3	UNKN D4	UNKN D5	UNKN D6	UNKN D7	UNKN D8	UNKN D9	UNKN D10	UNKN D11	UNKN D12
E	UNKN E1	UNKN E2	UNKN E3	UNKN E4	UNKN E5	UNKN E6	UNKN E7	UNKN E8	UNKN E9	UNKN E10	UNKN E11	UNKN E12
F	UNKN F1	UNKN F2	UNKN F3	UNKN F4	UNKN F5	UNKN F6	UNKN F7	UNKN F8	UNKN F9	UNKN F10	UNKN F11	UNKN F12
G	UNKN G1	UNKN G2	UNKN G3	UNKN G4	UNKN G5	UNKN G6	UNKN G7	UNKN G8	UNKN G9	UNKN G10	UNKN G11	UNKN G12
H	UNKN H1	UNKN H2	UNKN H3	UNKN H4	UNKN H5	UNKN H6	UNKN H7	UNKN H8	UNKN H9	UNKN H10	UNKN H11	UNKN H12

VIC Layer

The default layer for IPC assignments in the SDS v. 1.6.3 software is the JOE dye layer. These assigments must be changed to the VIC dye layer before using the Taqman Exogenous Internal Positive Control Reagents.

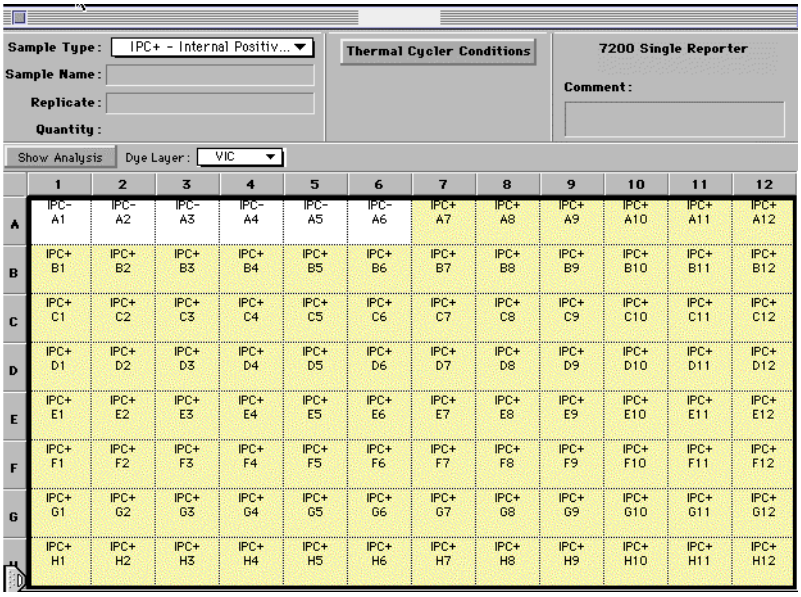
To set-up the IPC assignments for use with a VIC probe:

Step	Action
1	From the Dye Layer pop-up menu, select VIC.
2	From the Sample Type pop-up menu, select Sample Type Setup.  The SDS software displays the Sample Type Setup dialog box.
3	From the Internal Positive (IPC+) Reporter pop-up menu, select VIC.  The SDS software displays VIC in the Reporter box for the Internal Positive Control entry as shown below.  **IMPORTANT** The TaqMan VIC dye must be configured as a Pure Dye on the ABI PRISM® 7700/7200 Sequence Detection Systems for it to appear on the Reporter pull-down menu. See *User Bulletin #4: Generating New Spectra Components* (P/N 4306234) pages 6-7 to configure TaqMan VIC as a Pure Dye.
4	Repeat Step 3 to select VIC from the Internal Negative (IPC–) Reporter pop-up menu.
5	Click OK. The SDS software returns to the Setup Plate view.

Vic Layer Sample Type Setup

The following assignments should then be made in the Sample Type Setup to the VIC layer (see figure below):

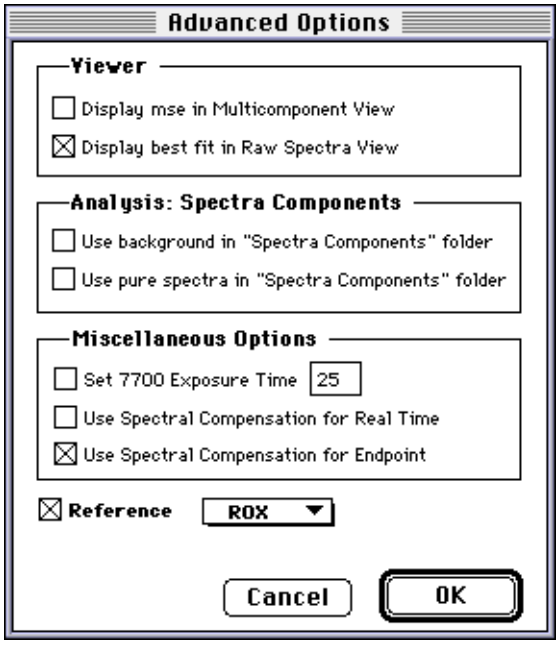
- Six Internal Positive Control Negative (IPC–) wells corresponding to the FAM layer NAC wells.
- Ninety Internal Positive Control Positive (IPC+) wells corresponding to the FAM layer NTC and UNKN wells.



Analyzing Data for End Point Runs

Analyzing Data

To analyze data:

Step	Action
1	Click the Show Analysis button on the setup window.
2	Click the Instrument/Advanced Options button. The Advanced Options dialog box appears. 
3	♦ If using the ABI PRISM 7700, select the “Use Spectral Compensation for End-Point” option. ♦ If using the ABI PRISM 7200, do not select the “Use Spectral Compensation for End-Point” option.
4	Click OK.
5	Click Analyze.
6	Click Display R_n from the Analysis menu.

To analyze data:

Step	Action
7	Examine the R_n values for the NTC wells in the FAM layer to confirm their reproducibility. **Note** These wells are used to calculate the target threshold value.
8	Examine the R_n values for the IPC– wells in the VIC layer to confirm their reproducibility. **Note** These wells are used to calculate the IPC threshold value.
9	Click the Window button.
10	Click the Event Log button.
11	Examine the Event Log to follow the process by which the SDS 1.6.3 software identifies outliers and generates threshold values.
12	Print the Experimental Report.

Note The FAM data from the NAC wells are not used in any calculations and usually these NAC wells are assigned No Amp. (This is designated by a "?" in the analysis plate view). In some instances, however, they may be assigned as target positive because of the addition of the IPC blocking solution to these wells. This does not represent a problem, and will not impact the correct assignment of unknown sample wells.

Target Template Calls

The ABI PRISM 7700 or 7200 Sequence Detectors determine positive (+) or negative (–) calls as described below. Refer to your instruments user's manual for more information.

If the Detectable Target Template (FAM) call is...	And the Detectable IPC (VIC) call is...	Then the Target Template is...
+	+, –[a]	+
–	+	–
–	–	No Amp / ?

a. In the presence of a strong FAM signal for the target assay, a negative assignment and/or signal can be obtained in the VIC layer. This results from the limiting primer concentrations used in the IPC assay.

Technical Support

Contacting Technical Support

You can contact Applied Biosystems for technical support by telephone or fax, by e-mail, or through the Internet. You can order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents 24 hours a day. In addition, you can download documents in PDF format from the Applied Biosystems Web site (please see the section “To Obtain Documents on Demand” following the telephone information below).

To Contact Technical Support by E-Mail

Contact technical support by e-mail for help in the following product areas:

Product Area	E-mail address
Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com
Sequence Detection Systems and PCR	pcrlab@appliedbiosystems.com
Protein Sequencing, Peptide and DNA Synthesis	corelab@appliedbiosystems.com
Biochromatography, PerSeptive DNA, PNA and Peptide Synthesis systems, CytoFluor®, FMAT™, Voyager™, and Mariner™ Mass Spectrometers	tsupport@appliedbiosystems.com
LC/MS (Applied Biosystems/MDS Sciex)	apisupport@sciex.com or api3-support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

Hours for Telephone Technical Support

In the United States and Canada, technical support is available at the following times:

Product	Hours
Chemiluminescence	8:30 a.m. to 5:30 p.m. Eastern Time
Framingham support	8:00 a.m. to 6:00 p.m. Eastern Time
All Other Products	5:30 a.m. to 5:00 p.m. Pacific Time

To Contact Technical Support by Telephone or Fax

In North America

To contact Applied Biosystems Technical Support, use the telephone or fax numbers given below. (To open a service call for other support needs, or in case of an emergency, dial **1-800-831-6844** and press **1**.)

Product or Product Area	Telephone Dial...	Fax Dial...
ABI PRISM® 3700 DNA Analyzer	**1-800-831-6844,** then press **8**	**1-650-638-5981**
DNA Synthesis	**1-800-831-6844**, then press **21**	**1-650-638-5981**
Fluorescent DNA Sequencing	**1-800-831-6844**, then press **22**	**1-650-638-5981**
Fluorescent Fragment Analysis (includes GeneScan® applications)	**1-800-831-6844**, then press **23**	**1-650-638-5981**
Integrated Thermal Cyclers (ABI PRISM® 877 and Catalyst 800 instruments)	**1-800-831-6844**, then press **24**	**1-650-638-5981**
ABI PRISM® 3100 Genetic Analyzer	**1-800-831-6844**, then press **26**	**1-650-638-5981**
BioInformatics (includes BioLIMS®, BioMerge™, and SQL GT™ applications)	**1-800-831-6844**, then press **25**	**1-505-982-7690**
Peptide Synthesis (433 and 43X Systems)	**1-800-831-6844**, then press **31**	**1-650-638-5981**
Protein Sequencing (Procise® Protein Sequencing Systems)	**1-800-831-6844**, then press **32**	**1-650-638-5981**
PCR and Sequence Detection	**1-800-762-4001**, then press **1** for PCR, **2** for the 7700 or 5700, **6** for the 6700 or dial **1-800-831-6844**, then press **5**	**1-240-453-4613**

Product or Product Area	Telephone Dial...	Fax Dial...
Voyager™ MALDI-TOF Biospectrometry and Mariner™ ESI-TOF Mass Spectrometry Workstations	**1-800-899-5858**, then press **13**	**1-508-383-7855**
Biochromatography (BioCAD® Workstations and Poros® Perfusion Chromatography Products)	**1-800-899-5858**, then press **14**	**1-508-383-7855**
Expedite™ Nucleic acid Synthesis Systems	**1-800-899-5858**, then press **15**	**1-508-383-7855**
Peptide Synthesis (Pioneer™ and 9050 Plus Peptide Synthesizers)	**1-800-899-5858**, then press **15**	**1-508-383-7855**
PNA Custom and Synthesis	**1-800-899-5858,** then press **15**	**1-508-383-7855**
FMAT™ 8100 HTS System and Cytofluor® 4000 Fluorescence Plate Reader	**1-800-899-5858**, then press **16**	**1-508-383-7855**
Chemiluminescence (Tropix)	**1-800-542-2369** (U.S. only), or **1-781-271-0045**	**1-781-275-8581**
Applied Biosystems/MDS Sciex	**1-800-952-4716**	**1-650-638-6223**

Outside North America

Region	Telephone Dial...	Fax Dial...
Africa and the Middle East		
Africa (English Speaking) and West Asia (Fairlands, South Africa)	**27 11 478 0411**	**27 11 478 0349**
South Africa (Johannesburg)	**27 11 478 0411**	**27 11 478 0349**
Middle Eastern Countries and North Africa (Monza, Italia)	**39 (0)39 8389 481**	**39 (0)39 8389 493**

Region	Telephone Dial...	Fax Dial...
Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	**61 3 9730 8600**	**61 3 9730 8799**
China (Beijing)	**86 10 64106608**	**86 10 64106617**
Hong Kong	**852 2756 6928**	**852 2756 6968**
Korea (Seoul)	**82 2 593 6470/6471**	**82 2 593 6472**
Malaysia (Petaling Jaya)	**60 3 758 8268**	**60 3 754 9043**
Singapore	**65 896 2168**	**65 896 2147**
Taiwan (Taipei Hsien)	**886 2 2358 2838**	**886 2 2358 2839**
Thailand (Bangkok)	**66 2 719 6405**	**66 2 319 9788**
Europe		
Austria (Wien)	**43 (0)1 867 35 75 0**	**43 (0)1 867 35 75 11**
Belgium	**32 (0)2 712 5555**	**32 (0)2 712 5516**
Czech Republic and Slovakia (Praha)	**420 2 61 222 164**	**420 2 61 222 168**
Denmark (Naerum)	**45 45 58 60 00**	**45 45 58 60 01**
Finland (Espoo)	**358 (0)9 251 24 250**	**358 (0)9 251 24 243**
France (Paris)	**33 (0)1 69 59 85 85**	**33 (0)1 69 59 85 00**
Germany (Weiterstadt)	**49 (0) 6150 101 0**	**49 (0) 6150 101 101**
Hungary (Budapest)	**36 (0)1 270 8398**	**36 (0)1 270 8288**
Italy (Milano)	**39 (0)39 83891**	**39 (0)39 838 9492**
Norway (Oslo)	**47 23 12 06 05**	**47 23 12 05 75**
Poland, Lithuania, Latvia, and Estonia (Warszawa)	**48 (22) 866 40 10**	**48 (22) 866 40 20**
Portugal (Lisboa)	**351 (0)22 605 33 14**	**351 (0)22 605 33 15**
Russia (Moskva)	**7 095 935 8888**	**7 095 564 8787**
South East Europe (Zagreb, Croatia)	**385 1 34 91 927**	**385 1 34 91 840**
Spain (Tres Cantos)	**34 (0)91 806 1210**	**34 (0)91 806 1206**
Sweden (Stockholm)	**46 (0)8 619 4400**	**46 (0)8 619 4401**
Switzerland (Rotkreuz)	**41 (0)41 799 7777**	**41 (0)41 790 0676**
The Netherlands (Nieuwerkerk a/d IJssel)	**31 (0)180 331400**	**31 (0)180 331409**

Region	Telephone Dial...	Fax Dial...
United Kingdom (Warrington, Cheshire)	**44 (0)1925 825650**	**44 (0)1925 282502**
All other countries not listed (Warrington, UK)	**44 (0)1925 282481**	**44 (0)1925 282509**
Japan		
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Appendix A. Preventing Contamination

Introduction

Due to the high throughput and repetitive nature of the 5´ nuclease assay, special laboratory practices are necessary in order to avoid false positive amplifications (Kwok and Higuchi, 1989). This is because of the capability for single DNA molecule amplification provided by the PCR process (Saiki *et al.*, 1985; Mullis *et al.*, 1987).

AmpErase UNG

AmpErase® UNG (uracil-N-glycosylase, UNG) is a pure nuclease-free, 26-kDa recombinant enzyme encoded by the *Escherichia coli* uracil-N-glycosylase gene. This gene has been inserted into an *E. coli* host to direct expression of the native form of the enzyme (Kwok and Higuchi, 1989).

UNG acts on single- and double-stranded dU-containing DNA. It acts by hydrolyzing uracil-glycosidic bonds at dU-containing DNA sites. The enzyme causes the release of uracil, thereby creating an alkali-sensitive apyrimidic site in the DNA. The enzyme has no activity on RNA or dT-containing DNA.

General PCR Practices

Use the following precautions to minimize sample cross-contamination and PCR product carryover:

- Wear a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation) and clean gloves when preparing samples for PCR amplification.
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Try not to splash or spray PCR samples.
- Use positive-displacement or air-displacement pipettors with filter-plugged tips. Change tips after each use.
- Keep reactions and components capped as much as possible.
- Clean lab benches and equipment periodically with 10% bleach solution.

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