ABI PRISM™ 7000 Sequence Detection System and Applied Biosystems™ 7500 Real-Time PCR System

Pub. No. 4374416 **Rev.** C

SUBJECT: **Instrument Calibration and Maintenance Procedures for Use with Quantifiler™ DNA Quantification Kits**

In This User Bulletin

Overview

Purpose

This bulletin provides Human Identification laboratories running Quantifiler™ DNA Quantification Kits on the ABI PRISM™ 7000 Sequence Detection System and the Applied Biosystems 7500 Real-Time PCR System with a comprehensive plan for instrument calibration and maintenance and recommended laboratory practices to assist in improving Quantifiler™ assay performance and preventing contamination.

The information in this bulletin supplements the specific documents available for use with Quantifiler™ Kits, the 7000 System running Sequence Detection System (SDS) Software v1.0 and v1.2.3, and the 7500 System running SDS Software v1.2.3 (see "Related Documentation" on page 2).

Safety

For safety guidelines, refer to the "Safety" section in the *ABI PRISM™ 7000 Sequence Detection System User Guide* and *Quantifiler™ Kits User's Manual,* and the "Safety and EMC Compliance" section in the *Applied Biosystems 7300/7500 Real-Time PCR System Installation and Maintenance Guide* and the *SDS Software Online Help*.

Related Documentation

For more detailed instructions on performing the calibrations and routine maintenance discussed throughout this user bulletin, refer to the documents listed below.

Product	Documentation
7000 System with SDS Software v1.0 and v1.2.3	*ABI PRISM™ 7000 Sequence Detection System User Guide* (PN 4330228)
7500 System with SDS Software v1.2.3	• *Applied Biosystems 7300/7500 Real-Time PCR System Installation and Maintenance Guide* (PN 4347828) • *SDS Software Online Help*‡
Quantifiler™ DNA Quantification Kits (PN 4343895, PN 4343906)	*Quantifiler™ Kits User's Manual* (PN 4344790)

‡ The online help is embedded in the SDS Software and does not require an Internet connection. To access the online help, select **Help Menu > Contents and Index** from the SDS Software menu bar.

How to Obtain HID Support

For Human Identification (HID)-specific support while running Quantifiler™ Kit assays on the 7000 System and the 7500 System, please use the contact information provided below.

By phone: 1-888-821-4HID (4443), select **Option 1**

By e-mail: HIDTechSupport@appliedbiosystems.com

Required Materials

The following table lists the user-supplied materials required to perform the instrument maintenance and calibration procedures discussed in this bulletin. Unless otherwise noted, several of the items are available from major laboratory suppliers (MLS).

Material	Source
Hardware/Equipment	
• 7000 System, Tungsten-halogen lamp (21V, 150W) • 7500 System, Halogen lamp (12V, 75W)	Applied Biosystems • (PN 4347754) • (PN 4345287)
Electronics fuses (12.5A, 250V, 5 × 20MM)	MLS

Material	Source
Phillips and flathead screwdrivers	MLS
Reagents	
TaqMan™ RNase P Instrument Verification Plate: • 7000 System • 7500 System	Applied Biosystems • (PN 4310982) • (PN 4350584)
Spectral Calibration Kit: • 7000 System (8 preloaded and sealed calibration plates: 1 Background, 7 Spectral) • 7500 System (9 preloaded and sealed calibration plates: 1 Background, 1 Region of Interest (ROI), 7 Spectral)	Applied Biosystems • (PN 4328895) • (PN 4349180)
10% bleach solution	MLS
95% ethanol solution	MLS
Consumables	
Optical 96-Well Reaction Plates (500 plates)	Applied Biosystems (PN 4316813)
Optical Adhesive Covers Starter Kit (20 covers, 1 compression pad, 1 applicator)	Applied Biosystems (PN 4313663)
Optical Tubes (8 tubes/strip, 125 strips)	Applied Biosystems (PN 4316567)
Optical Caps (8 flat caps/strip, 300 strips)	Applied Biosystems (PN 4323032)
MicroAmp™ Cap Installing Tool: • Roller • Handle	Applied Biosystems • (PN N8010438) • (PN 4330015)
Precision Plate Holder for the 7000 System	Applied Biosystems (PN 4350105)
Powder-free gloves	MLS
Lint-free cotton swabs	MLS
Safety glasses	MLS

Instrument Maintenance Procedures

Recommended Maintenance Schedule

The 7000 System, the 7500 System, and the computer that runs the instrument need to be properly and routinely maintained to ensure optimum performance. Applied Biosystems recommends that you perform specific instrument maintenance tasks according to the schedule outlined in the table below.

Note: Refer to the *ABI PRISM™ 7000 Sequence Detection System User Guide* or the *Applied Biosystems 7300/7500 Real-Time PCR System Installation and Maintenance Guide* for more detailed maintenance procedures.

Schedule	Maintenance Task
Weekly	• Archive and back-up your computer files. • Restart the computer. • Wipe instrument surfaces with a lint-free cloth.
Monthly	• Defragment the computer hard drive. • Perform the System Hardware Test (Function-Test). • Check sample wells for contamination. **Note:** You can identify contaminated sample wells by either performing a background calibration (see "Recommended Calibration Schedule" on page 12) or using the ROI Inspector to take an image of the thermal cycler sample block. Acceptable signal ranges for the background calibration are < 1,200 Fluorescent Standard Units (FSU) for the 7000 System and < 72,000 FSU for the 7500 System (< 90,000 FSU for filter set E). If contaminated wells are detected, clean the sample wells and recheck them for contamination.
As Needed	• Clean the sample wells. • Replace the halogen lamp. **IMPORTANT!** You must perform the following calibrations *in this order* after successfully replacing the halogen lamp: ROI > background > optical (*7500 System only*) > pure dye > instrument verification. • Replace the instrument fuses.

How to Maintain the Halogen Lamp

Fluorescence detection is accomplished in the 7000 System and the 7500 System by a halogen lamp, which directs light to each well of a mounted reaction plate and excites the fluorescent dyes therein. The average lifetime of the installed halogen lamp is approximately 2000 hours on the 7500 System and 1000 hours on the 7000 System. To help maintain the lamp, please keep in mind the following:

- The lamp remains illuminated while the instrument is powered on. To increase the lifetime of the lamp, power down the instrument when not in use.

  Note: The 7500 System will automatically turn off the halogen lamp if the instrument remains unused for ≥ 4 hours.

- Allow at least 15 minutes for the lamp to cool before removing it from the instrument.

  PHYSICAL INJURY HAZARD. The lamp can become very hot while in use. Allow sufficient time for the lamp to cool, and put on protective gloves before handling it.

- Wear powder-free gloves when handling the lamp.

When to Replace the Halogen Lamp

You should replace the halogen lamp:

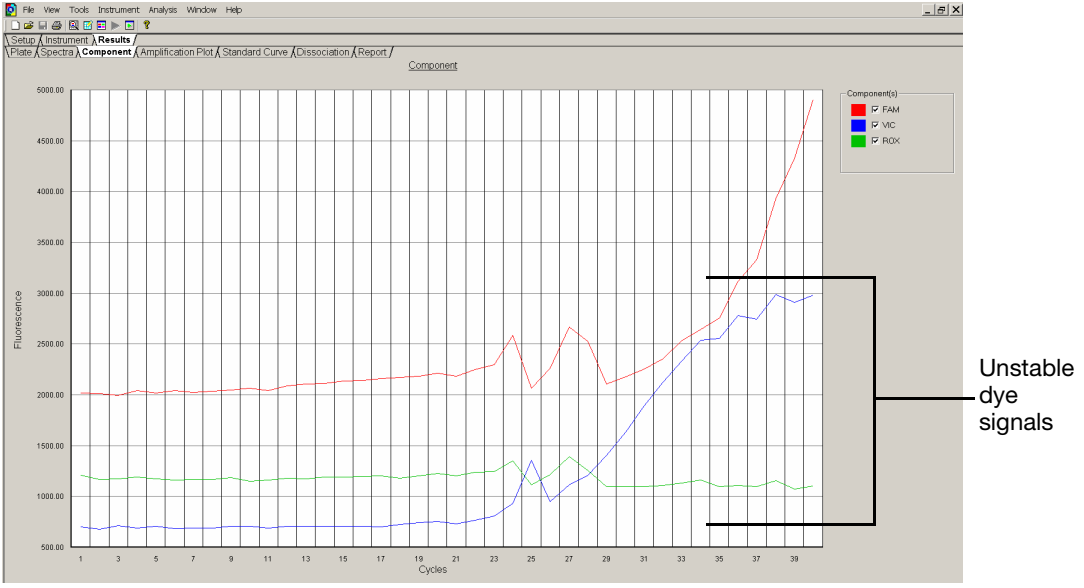
- After approximately 2000 hours of use on the 7500 System and 1000 hours of use on the 7000 System.
- If unstable signals are generated in all filters (component dyes) during a run. See "How to View Component Dye Signals" on page 7 for instructions on how to use the SDS Software to view these signals.
- If the instrument fails to produce any raw fluorescence data.
- If the standard curve consistently fails to meet specifications when the quality of the standards themselves is ruled out as a factor.

  Note: Refer to the *Quantifiler™ Kits User's Manual* for more information on examining the standard curve results.

If one or more of the above conditions are true for your system, check the lamp status to determine whether the lamp needs to be replaced. See "How to Check Lamp Status" on page 8 for the lamp status monitoring procedure appropriate to your system.

How to View Component Dye Signals

To view the component dye signals from a Quantifiler™ Kit run, select the **Component** sub-tab within the Results view of the SDS Software. The halogen lamp may need replacement if the lines representing the dye signals contain spikes or appear unstable, as shown in the figure below. Applied Biosystems does not use the dye signals found in the Component sub-tab as a measure of lamp life or need for replacement.



IMPORTANT! This signal determination *may* be implemented as a routine procedure to monitor the life of the halogen lamp. However, it is critical that the reading is taken from the *same* sample well to provide a better comparison between determinations.

How to Check Lamp Status

Checking Lamp Function

To check for proper lamp function, select **Instrument > Function-Test** from the SDS Software menu bar. The Function-Test dialog displays a lamp function status message as shown in the following table.

If the lamp status message is...	Then...
Pass	• For the 7500 System: – The lamp is functioning well. There is no need to replace the bulb at this time. • For the 7000 System: – Perform a visual inspection of the lamp to make sure the lamp remains illuminated while the instrument is powered on (see "Checking for Proper Lamp Illumination" on page 11).
Fail	• For the 7500 System only: – Check the lamp current using the Lamp Status/Replacement function. See "Checking the Lamp Current (7500 System only)" on page 9 for more information. • For the 7500 System or the 7000 System, you can choose to either: – Perform a visual inspection to verify proper lamp illumination. See "Checking for Proper Lamp Illumination" on page 11 for more information. *or* – Replace the halogen lamp. Refer to the *ABI PRISM™ 7000 Sequence Detection System User Guide* or the *Applied Biosystems 7300/7500 Real-Time PCR System Installation and Maintenance Guide* for the proper procedure.

Checking the Lamp Current (*7500 System only*)

To check whether the halogen lamp on the 7500 System has enough electrical current, select **Instrument > Lamp Status/Replacement** from the SDS Software menu bar. The Lamp Status/Replacement dialog displays the lamp usage (hours), current (percent), and one of three lamp status messages as shown in the table on the next page.

If the lamp status is...	Then...
Good	The lamp is functioning well. There is no need to replace the bulb at this time.
Change Soon	The lamp bulb usage is either above 2000 hours, or it may be dim but functional. Applied Biosystems recommends that you replace the lamp soon.
Failed	The lamp bulb must be replaced. Refer to the *Applied Biosystems 7300/7500 Real-Time PCR System Installation and Maintenance Guide* for the proper procedure.

Note: The SDS Software for the 7500 System also displays warning messages before or during a run that indicate low lamp current. Refer to the *Applied Biosystems 7300/7500 Real-Time PCR System Installation and Maintenance Guide* for more information regarding these messages.

Checking the Performance of the Replacement Lamp

After you replace the halogen lamp, run the Function-Test and/or the Lamp Status/Replacement function (*7500 System only*) again to ensure proper lamp performance. Follow the appropriate actions for the performance results as shown in the table below.

If the...	Then...
• Function-Test fails *or* • Lamp/Status Replacement Test fails (*7500 System only*)	The lamp may not be properly illuminated (see "Checking for Proper Lamp Illumination" on page 11) and/or the fuses may need to be replaced. **Note:** Refer to the *ABI PRISM™ 7000 Sequence Detection System User Guide* or the *Applied Biosystems 7300/7500 Real-Time PCR System Installation and Maintenance Guide* for the proper fuse replacement procedure.
• Function-Test passes (*7000 System only*)	Perform a visual inspection of the lamp to make sure the lamp remains illuminated while the instrument is powered on (see "Checking for Proper Lamp Illumination" on page 11).
• Lamp/Status Replacement Test passes (*7500 System only*)	The lamp is functioning well. Click **Reset Lamp Timer** in the Lamp Status/Replacement dialog to reset the lamp usage counter in the SDS Software.

Checking for Proper Lamp Illumination

After you replace the halogen lamp, perform a visual inspection to verify proper lamp illumination. For both the 7000 System and the 7500 System, the lamp remains illuminated while the instrument is powered on.

To check lamp illumination on the 7000 System:

1.	Lift open the lamp access door located on the top of the instrument.
2.	Turn on the 7000 System.
3.	While the instrument is running, look through the access door opening to verify whether the lamp is illuminated.

To check lamp illumination on the 7500 System:

1.	Make sure the access door is closed.
2.	Turn on the 7500 System.
3.	In the SDS Software, select **Instrument > Calibrate**.
4.	In the ROI Inspector dialog box, select **Lamp Control > Idle**.
5.	While the instrument is running, look through the grating of the access door to verify whether the lamp is illuminated, then click **Done**.

If the halogen lamp is not illuminated, the replacement halogen lamp may be defective. Replace the lamp again and repeat the visual inspection. If the second lamp does not illuminate, check the instrument fuses for failure. Refer to the *ABI PRISM™ 7000 Sequence Detection System User Guide* or the *Applied Biosystems 7300/7500 Real-Time PCR System Installation and Maintenance Guide* for the proper procedure.

Note: If the lamp still does not illuminate after the replacement of the instrument fuses, please contact your local Service Engineer, HID Technical Support representative or HID Application Specialist (see "How to Obtain HID Support" on page 2 for contact information).

IMPORTANT! Perform the following calibrations *in this order* after successfully replacing the lamp: ROI > background > optical (*7500 System only*) > pure dye > instrument verification.

Instrument Calibration Procedures

Recommended Calibration Schedule

The 7000 System and the 7500 Systems need to be properly and routinely calibrated to ensure optimum instrument and assay performance. Applied Biosystems recommends that you perform specific instrument calibration tasks according to the schedule outlined in the table below.

Note: Refer to the *ABI PRISM™ 7000 Sequence Detection System User Guide* or the *Applied Biosystems 7300/7500 Real-Time PCR System Installation and Maintenance Guide* for more detailed calibration procedures.

Schedule	Calibration Task
Monthly	• Perform a background calibration.
Every 6 Months	• Perform an ROI calibration. • Perform a background calibration. • Perform an optical calibration (*7500 System only*). • Perform a Pure Dye Spectra calibration. **IMPORTANT!** You must also perform the following calibrations *in this order* after successfully replacing the halogen lamp: ROI > background > optical (*7500 System only*) > pure dye > instrument verification.

Verifying Instrument Performance

About the TaqMan™ RNase P Instrument Verification Plate

The TaqMan™ RNase P Instrument Verification Plate is used to verify that the instrument meets performance specifications. A Service Engineer performs this test at the time of instrument installation after all calibrations have been performed.

Running the TaqMan™ RNase P Verification Plate (*optional*)

Laboratories may choose to implement this procedure as part of their routine maintenance. You can also use this test as a troubleshooting tool to identify whether a problem your laboratory is encountering is due to a chemistry or an instrument issue.

To run this test, the TaqMan™ RNase P Verification Plate must be purchased from Applied Biosystems. The instructions for running the plate and analyzing the data can be found on the product insert that comes inside the box containing the plate. Please refer to the table below for the appropriate TaqMan™ RNase P Verification Plate Kit part numbers for your instrument.

Instrument	Kit PN	Description
7000 System	4310982	Plate kit includes one MicroAmp™ Optical 96-Well Reaction Plate pre-loaded and sealed with complete TaqMan™ reagents to detect and quantitate genomic copies of the human RNase P gene.
7500 System	4350584	

Improving Assay Performance

About Assay Sensitivity

Real-Time PCR assays are extremely sensitive and detection of C_T values greater than 35 may indicate the presence of exceedingly low quantities of DNA (< 3 copies). Some user laboratories have reported the detection of C_T values < 40 for extraction blank and negative control samples when performing the Quantifiler™ Kit assays. Applied Biosystems has confirmed that detection of such a low quantity of DNA may vary from amplification to amplification based on stochastic effects. Such levels may be considered "background" and will not produce detectable product when the AmpFℓSTR™ Kits are used.

The Quantifiler™ Kit reagents undergo rigorous quality control to ensure that the reagents are free of contaminants. However, due to the extreme sensitivity of the test, background DNA from the environment may be detected on rare occasions. Each laboratory should take the usual precautions to minimize contamination in their own facility and establish a C_T value above which a positive result represents only background DNA. In this way, samples that can be successfully amplified using the AmpFℓSTR™ Kits can be distinguished from those samples lacking sufficient target to generate a meaningful result.

Establishing the limits of the test is common practice in forensic laboratories when dealing with STR amplification results, and Applied Biosystems recommends applying a similar approach when dealing with results generated by the Quantifiler™ Kit.

About False Positive Results

It is important to note the following when encountering false positive results (positive amplification of negative controls):

- The quantities obtained are usually well below the dynamic range of the standard curve. Therefore, these quantities cannot be used accurately.
- You may choose to lower the cycle number to overcome such high level sensitivity.

  Note: Lowering the cycle number would require further internal validation at your facility.

- You may choose to set a C_T value threshold for proceeding with STR analysis.

  Note: Setting a C_T value threshold would require further internal validation at your facility.

Preventing PCR Contamination

PCR assays require special laboratory practices to avoid false positive amplifications. The high sensitivity of these assays can lead to amplification of a single DNA molecule.

To minimize false positives due to amplified material in your work area, follow these recommended laboratory practices:

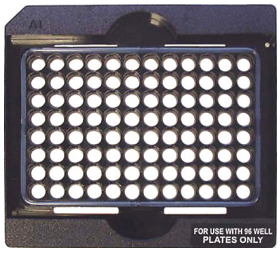
- If possible, maintain separate work areas, dedicated equipment, and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Wear a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation) and clean gloves when preparing samples for PCR amplification.
- Change gloves whenever you suspect that they are contaminated and before leaving the work area.
- Use positive-displacement pipettes or aerosol-resistant pipette tips.
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes and reaction plates carefully. Try not to splash or spray PCR samples.
- When pipetting from a kit component tube, hold the cap of the tube in your gloved hand, or be sure to set it down on a clean, decontaminated surface.
- Keep reactions and components sealed as much as possible.
- Do not open sealed reaction tubes or plates after amplification.
- Clean lab benches and equipment periodically with freshly diluted 10% bleach solution.

Improving Replicate Precision

The Precision Plate Holders for the 7000 System and the 7500 System improve thermal cycler performance and the precision of replicates by reducing corner well condensation. The Precision Plate Holder fits between individual reaction tubes, tube strips, and 96-well reaction plates (including the TaqMan™ RNase P Verification Plate) and the thermal cycler sample block.

Note: Applied Biosystems recommends using the Precision Plate Holder with every 7000 System and 7500 System run.

Please refer to the table below for the appropriate Precision Plate Holder for your instrument and run configuration.

For the...	Running...	Use Precision Plate Holder...
7000 System	• 96-Well Reaction Plates • Tube Strips • Individual Tubes	PN 4350105‡ 
7500 System	• 96-Well Reaction Plates	
	• Tube Strips • Individual Tubes	

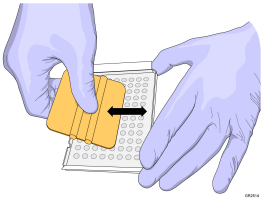
‡ Use the Precision Plate Holder on the 7000 System with the recessed and notched side facing *up* to ensure proper placement and release of individual reaction tubes, tube strips, and 96-well reaction plates from the thermal cycler sample block.

Note: If you do not have the proper Precision Plate Holder for your instrument or run configuration, please contact your local Service Engineer, HID Technical Support representative or HID Application Specialist (see "How to Obtain HID Support" on page 2 for contact information).

Preventing Sample Loss

A tight reaction plate or tube seal provides consistent results and prevents evaporation from and/or condensation within the sample wells (sample loss) as well as well-to-well contamination. Follow the steps below to ensure a proper seal.

To seal optical plates:

1.	Remove the *middle* portion of the protective backing of an optical adhesive cover and hold the cover by the perforated end tabs, taking care not to touch the *underside* of the cover.
2.	Place the optical adhesive cover on the plate, then rub the flat edge of the applicator back and forth along the long edge (*length*) of the plate. **IMPORTANT!** Apply significant downward pressure on the applicator in all steps to form a complete seal on top of the wells. Pressure is required to activate the adhesive on the optical cover. 
3.	Rub the flat edge of the applicator back and forth along the short edge (*width*) of the plate. 
4.	Rub the end of the applicator horizontally and vertically between all wells.
5.	Rub the end of the applicator around all *outside* edges of the plate using small back and forth motions to form a complete seal around the outside wells. 

To seal optical plates: ***(continued)***

6.	Use the applicator to apply pressure to the perforated end tabs of the optical adhesive cover, then remove the tabs from the cover's protective backing prior to amplification. **Note:** Applied Biosystems recommends you place a compression pad on the sealed reaction plate before you insert it into the thermal cycling sample block on the 7000 System. **IMPORTANT!** Do not use a compression pad with the 7500 System.

To seal tubes:

1.	Place strip caps on the tubes. **Note:** To increase the quality of your results, Applied Biosystems recommends that you place the reaction tubes toward the center of the reaction tray rather than the edges.
2.	**IMPORTANT!** Apply significant downward pressure on the capping tool in all steps to form a complete seal on top of the tubes. If you are using the roller capping tool (PN N8010438): • Roll the capping tool across all strips of caps on the short edge, then the long edge, of the tray. • Roll the capping tool around all outer rows of strips of caps.  If you are using the handle capping tool (PN 4330015): • Slip your fingers through the handle with the holes in the tool facing down. • Place the holes in the tool over the first eight caps in a row. • Rock the tool back and forth a few times to seal the caps. • Repeat for remaining caps in the row, then for all remaining rows. 

Notes:

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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Revision history: Pub. No. 4374416

Revision	Date	Description
C	24 August 2018	Updated branding and trademarks, no technical changes.
B	April 2007	Basis for this revision.

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