

TECHNICAL MANUAL

ProNex[®] DNA QC Assay Calibration Kit, 7500

Instructions for Use of Products
NG1001



ProNex[®] DNA QC Assay Calibration Kit, 7500

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1. Description

The ProNex[®] DNA QC Assay evaluates the quantity and quality of genomic DNA extracted from formalin-fixed paraffin-embedded (FFPE) samples or other potentially degraded DNA sources. It is a human-specific, multiplexed probe-based quantitative polymerase chain reaction (qPCR) assay that may also be used to evaluate the ratio of circulating cell-free DNA (ccfDNA) to higher molecular weight genomic DNA in plasma samples. The multiplex assay detects 75bp, 150bp and 300bp human genomic DNA sequences, and it includes an internal positive control (IPC) to test for false-negative results that may occur in the presence of PCR inhibitors. This technical manual describes the ProNex[®] DNA QC Assay Dye Calibration Kit, 7500 and provides instructions for use with the Applied Biosystems[®] (ABI) 7500 and 7500 Fast Real-Time PCR System (Applied Biosystems[®] 7500 Software, Version 2.0.6 or newer) (1). If you have questions about compatibility with other instruments, please contact Promega Technical Services. Email: techserv@promega.com.

Before using the ProNex[®] DNA QC Assay on an ABI 7500/7500 Fast instrument, you must calibrate the instrument for FAM[™], CAL Fluor[®] Gold 540, TAMRA[™], Quasar[®] 670 and CXR dyes using the ProNex[®] DNA QC Assay Calibration Kit, 7500. To distinguish these dyes from dyes with the same name already calibrated on your instrument and for compatibility with the ProNex[®] DNA QC Analysis Software, use the following dye names: DNAQC_FAM (for FAM[™]), DNAQC_CFG540 (for CAL Fluor[®] Gold 540), DNAQC_TMR (for TAMRA[™]), DNAQC_CXR (for CXR), DNAQC_Q670 (for Quasar[®] 670).



1. Description (continued)

The dyes in the ProNex[®] DNA QC Assay Calibration Kit, 7500 are supplied at 100X concentration. The ProNex[®] DNA QC Calibration Buffer is supplied as a diluent for preparing a spectral calibration plate for each dye.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
ProNex [®] DNA QC Assay Calibration Kit, 7500	1 kit	NG1001

For Research Use Only. Not for use in diagnostic procedures. Includes:

- 1 × 60µl ProNex[®] DNA QC Calibration Standard, FAM
- 1 × 60µl ProNex[®] DNA QC Calibration Standard, CFG540
- 1 × 60µl ProNex[®] DNA QC Calibration Standard, TAMRA
- 1 × 60µl ProNex[®] DNA QC Calibration Standard, CXR
- 1 × 60µl ProNex[®] DNA QC Calibration Standard, Q670
- 1 × 30ml ProNex[®] DNA QC Calibration Buffer

ProNex[®] DNA QC Calibration Buffer is formulated to be used with calibration dyes for the ProNex[®] DNA QC Assay.

Storage conditions: Store the ProNex[®] DNA QC Calibration Kit, 7500 at –30°C to –10°C in a non-frost-free freezer. For short-term storage (less than 1 week), the kit can be stored at 2–10°C. Minimize the number of freeze-thaw cycles. The ProNex[®] DNA QC Assay Calibration Kit, 7500 is light-sensitive and must be stored in the dark.

3. Materials Required

Materials to Be Supplied by the User

- Applied Biosystems[®] 7500 or 7500 Fast Real-Time PCR System (Software Version 2.0.6 or newer must be used)
- MicroAmp[®] optical 96-well reaction plates for the Applied Biosystems[®] 7500 Real-Time PCR System (ABI Cat.# N8010560, 4316813, 4306737 or 4326659); OR
- MicroAmp[®] Fast optical 96-well reaction plates, 0.1ml for the Applied Biosystems[®] 7500 Fast Real-Time PCR System (ABI Cat.# 4346907, 4346906 or 4366932)
- MicroAmp[®] optical adhesive film (ABI Cat.# 4311971)
- MicroAmp[®] 96-well Support Base (ABI Cat.# 4379590)
- sterile, aerosol-resistant pipette tips
- tubes (5ml or larger)
- vortex mixer
- microplate centrifuge

Note: For support of other instruments with similar fluorescent dye capabilities, contact Promega Technical Services for more information. Email: techserv@promega.com.

4. General Considerations

- The ProNex® DNA QC Calibration Standards are light-sensitive and must be stored in the dark.
- We recommend performing the Regions of Interest (ROI) calibration, background calibration and optical calibration as described in the Applied Biosystems® 7500/7500 Fast Real-Time PCR Systems Maintenance Guide (1) before performing the ProNex® DNA QC Assay Calibration.
- The Applied Biosystems® 7500/7500 Fast Real-Time PCR Systems Maintenance Guide recommends performing custom pure dye calibrations at least every 6 months.
- Performing the dye calibrations will take approximately 1 hour (total).
- After calibration, store the calibration plates protected from light at –20°C for up to 4 months. Reuse the plates when reanalysis is necessary.

5. Plate Setup and Calibration Protocol

5.A. Plate Setup

1. Thaw the five ProNex® DNA QC Calibration Standards (FAM, CFG540, TAMRA, CXR and Q670) and ProNex® DNA QC Calibration Buffer.
2. Vortex each ProNex® DNA QC Calibration Standard and ProNex® DNA QC Calibration Buffer for 10 seconds to mix. Do not centrifuge the ProNex® DNA QC Calibration Standard after mixing.
3. Dilute each ProNex® DNA QC Calibration Standard 100-fold in ProNex® DNA QC Calibration Buffer in a separate 5ml or larger tube as described in Table 1.

Table 1. Preparation of Diluted ProNex® DNA QC Calibration Standards.

Reagent	FAM	CFG540	TAMRA	CXR	Q670
ProNex® DNA QC Calibration Standard, FAM	22µl	–	–	–	–
ProNex® DNA QC Calibration Standard, CFG540	–	22µl	–	–	–
ProNex® DNA QC Calibration Standard, TAMRA	–	–	22µl	–	–
ProNex® DNA QC Calibration Standard, CXR	–	–	–	22µl	–
ProNex® DNA QC Calibration Standard, Q670	–	–	–	–	22µl
ProNex® DNA QC Calibration Buffer	2,178µl	2,178µl	2,178µl	2,178µl	2,178µl
Total Volume	2,200µl	2,200µl	2,200µl	2,200µl	2,200µl



5.A. Plate Setup (continued)

4. Vortex each diluted ProNex[®] DNA QC Calibration Standard for 10 seconds to mix. Do not centrifuge the diluted standards after mixing.
5. Reserve a separate MicroAmp[®] optical 96-well reaction plate for each ProNex[®] DNA QC Calibration Standard. Record the bar code number or label the side of the plate skirt to indicate which calibration standard is in the plate.

Note: Wear gloves at all times when handling the plate, and take care to avoid touching the plate wells and MicroAmp[®] optical adhesive film unnecessarily. Handle the plate by the edges, and avoid touching the top or bottom of the plate. Use a MicroAmp[™] 96-Well Support Base to minimize contaminants on the bottom of the 96-well reaction plate.

6. Dispense 20 μ l of diluted ProNex[®] DNA QC Calibration Standard, FAM, into all 96 wells of the plate reserved for the FAM dye. Repeat this step for each of the other ProNex[®] DNA QC Calibration Standards by adding 20 μ l of the diluted calibration standard to each well of the appropriate dye-specific plate.
7. Seal each plate with MicroAmp[®] optical adhesive film. Protect the plates from exposure to light.
8. Centrifuge the plates briefly. To complete the calibration, proceed to Section 5.B.

5.B. Calibration

This protocol is intended for use with the Applied Biosystems[®] 7500 Real-Time PCR System and Applied Biosystems[®] 7500 Software (Version 2.0.6).

1. Turn on the computer associated with the Applied Biosystems[®] 7500 or 7500 Fast Real-Time PCR System.
2. Turn on the Applied Biosystems[®] 7500 or 7500 Fast Real-Time PCR System.
3. Launch the Applied Biosystems[®] 7500 Software, Version 2.0.6 (or newer).
4. From the Instrument menu at the top of the screen, select "Instrument Maintenance Manager".
5. Select the "Dye" section from the left panel.
6. Select "Custom Dye Calibration", and choose "Start Calibration" (Figure 1).

Perform a dye calibration to use fluorescent dyes in your experiments.
Dye The software uses the dye calibration data to determine the contribution of each fluorescent dye in the reactions.
 To perform a dye calibration, select the type of dye calibration to perform (system dye or custom dye), then click "Start Calibration."

Dye Calibration

System Dye Calibration
 Custom Dye Calibration

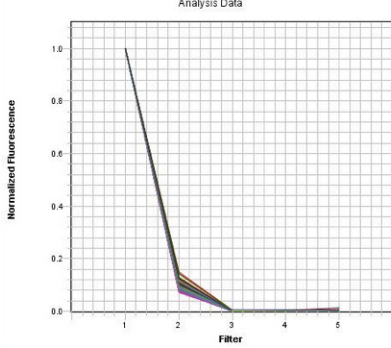
Dye Calibration Results

Dye	Type	Status	Last Run	Expiration Date	Temperature(°C)
DNAQC_CFG540	Custom	Current	2/3/16 3:17 PM	8/3/16 3:17 PM	60.0
DNAQC_CXR	Custom	Current	2/3/16 3:40 PM	8/3/16 3:40 PM	60.0
DNAQC_FAM	Custom	Current	2/3/16 3:09 PM	8/3/16 3:09 PM	60.0
DNAQC_0670	Custom	Current	2/3/16 3:32 PM	8/3/16 3:32 PM	60.0
DNAQC_TMR	Custom	Current	2/3/16 3:25 PM	8/3/16 3:25 PM	60.0

Dye Calibration Data

View Details for: Custom Plate (DNAQC_FAM)

Analysis Data



View Plate Layout | View Well Table

Show in Wells | View Legend

	1	2	3	4	5	6	7	8	9	10	11	12
A	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...
B	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...
C	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...
D	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...
E	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...
F	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...
G	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...
H	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...	DNA...

141467A

Figure 1. The Custom Dye Calibration option and Start Calibration button.

5.B. Calibration (continued)

7. In the Dye Calibration window that appears, select the “New Dye” button (Figure 2).

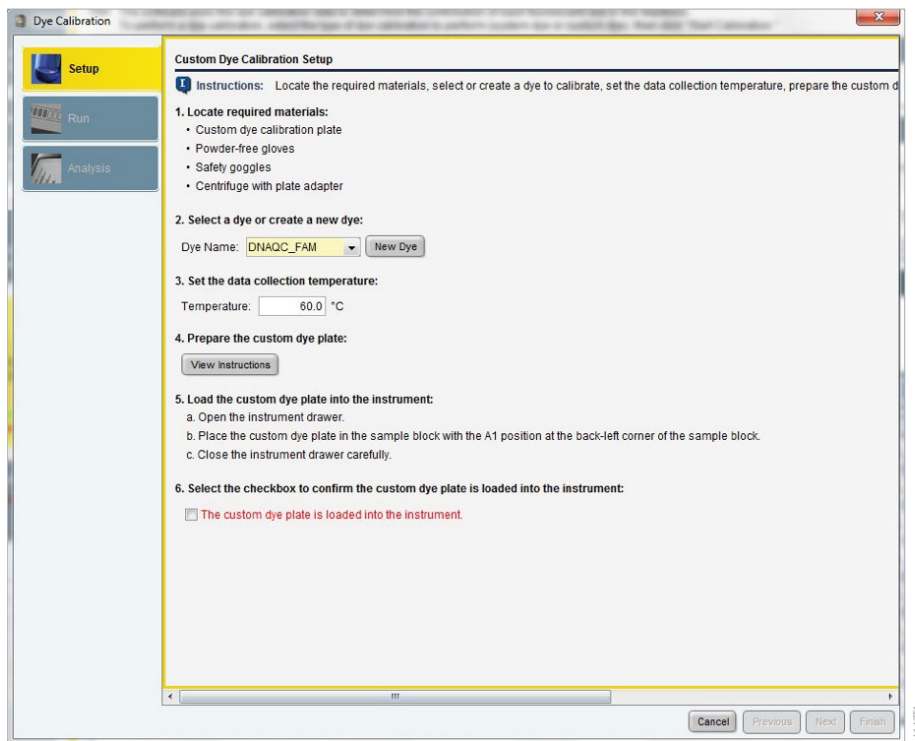


Figure 2. Calibrating a new dye.

- In the Dye Library window that appears, select the “New” button (Figure 3).

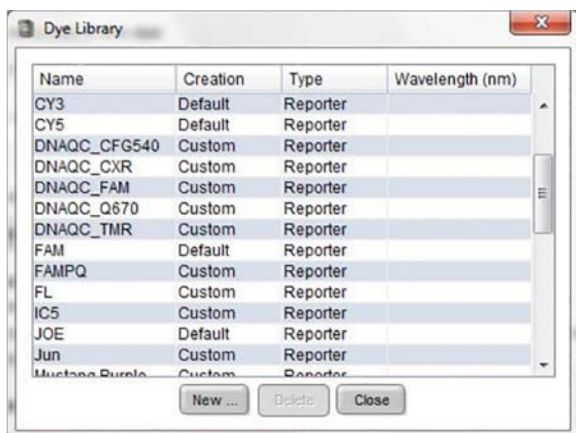


Figure 3. The New button in the Dye Library window.

- Enter “DNAQC_FAM” as the new dye name, select the “Reporter” radio button under “Type” and then select “OK” (Figure 4). Repeat this process to define the CFG540 (define as DNAQC_CFG540), TAMRA (define as DNAQC_TMR), CXR (define as DNAQC_CXR) and Q670 (define as DNAQC_Q670) dyes. For compatibility with the ProNex® DNA QC Analysis Software, use the recommended dye names.

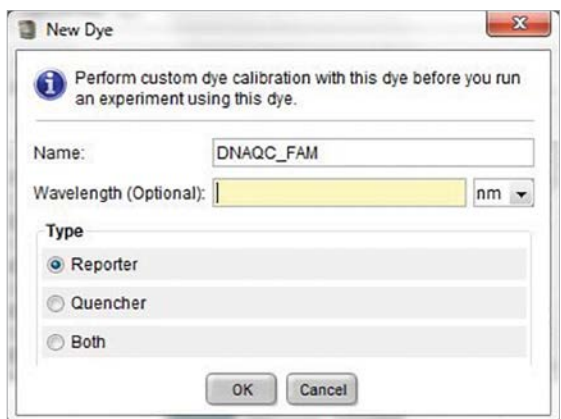


Figure 4. Naming the new dye.

5.B. Calibration (continued)

- In the Custom Dye Calibration Setup window, select “DNAQC_FAM” from the Dye Name drop-down list (Figure 5).

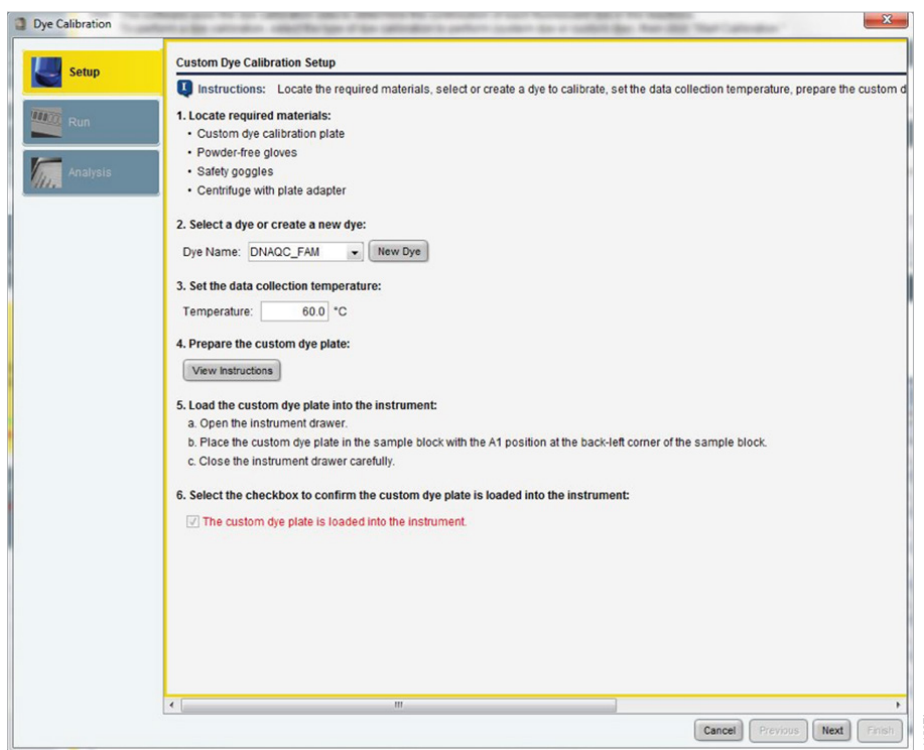


Figure 5. Selecting the dye name from the drop-down list.

- Load the plate prepared in Section 5.A for the ProNex[®] DNA QC Calibration Standard, FAM, into the instrument.
- Check the box for “The custom dye plate is loaded into the instrument” (Figure 5).
- Select “Next”, then “Start Run”.
- Once the calibration run is complete, select “Next” in the bottom right corner of the window. The software will display the spectra and indicate whether the spectra are acceptable (i.e., which spectra passed) under “Status”.
- Select “Finish”, and repeat steps 10 through 14 with each plate of ProNex[®] DNA QC Calibration Standards to calibrate the other dyes: CFG540 (DNAQC_CFG540), TAMRA (DNAQC_TMR), CXR (DNAQC_CXR) and Q670 (DNAQC_Q670).

Note: FAM should give highest signal in Filter 1, CFG540 in Filter 2, TAMRA in Filter 3, CXR in Filter 4 and Q670 in Filter 5. Figure 1 shows an example of a dye calibration spectrum (DNAQC_FAM) across all five filters. See Appendix, Section 7.B for additional figures showing typical spectrum for the other dyes.

6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: www.promega.com.

Symptoms	Causes and Comments
The custom dye calibration failed	Prepare new standard calibration plate(s) and recalibrate as described in Section 5.
Spectrum below the detectable threshold limit for the calibration	<ul style="list-style-type: none"> • Calibration dye plate was centrifuged insufficiently. • Calibration dye plate contains old or degraded dye reagents. • Calibration dye was over-diluted in preparation. Centrifuge the plate to confirm the liquid in the calibration dye plate is at the bottom of the well and of equal volume across the plate. Prepare new calibration dye plate(s) and recalibrate.
Spectral peak in the wrong filter	Confirm the correct dye plate was run during calibration. Repeat the dye calibration with the correct dye.
Spectrum contains peaks in more than one filter	The dye plates or thermal block could have fluorescent contaminants on them. Perform a background calibration to determine if contaminants are present on the thermal block. Clean the block if necessary.

For support of other instruments with similar fluorescent dye capabilities, please contact Promega Technical Services for more information. Email: techserv@promega.com.

7. Appendix

7.A. References

1. Applied Biosystems® 7500/7500 Fast Real-Time PCR Systems Maintenance Guide (2010) Part Number 4387777. Applied Biosystems, Foster City, CA.

7.B. Typical Dye Spectrum Images

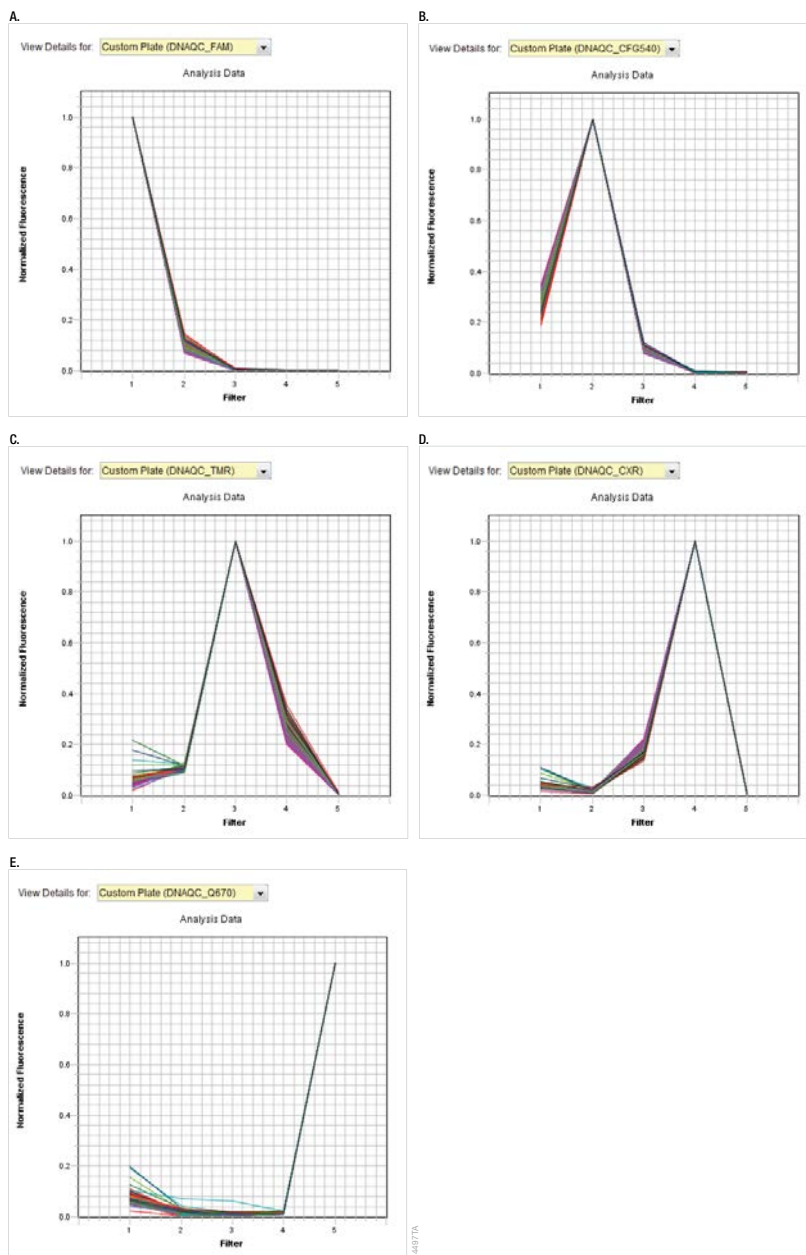


Figure 6. Dye Calibration Data. Panel A. Filter 1, FAM. **Panel B.** Filter 2, CFG540. **Panel C.** Filter 3, TAMRA. **Panel D.** Filter 4, CXR. **Panel E.** Filter 5, Q670.

7.C. Related Products

Product	Size	Cat.#
ProNex® DNA QC Assay ABI 7500/7500FAST	200 reactions	NG1002
	800 reactions	NG1003
ProNex® DNA QC Assay BioRad CFX96™	200 reactions	NG1004
	800 reactions	NG1005
ProNex® DNA QC Assay Software		7002422

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