

QIAxcel[®] DNA Handbook

QIAxcel DNA High Resolution Kit

QIAxcel DNA Screening Kit

QIAxcel DNA Fast Analysis Kit

For automated analysis of DNA fragments
using the QIAxcel and QIAxcel Advanced
instruments



QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

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Kit Contents

QIAxcel DNA High Resolution Kit	(1200)
Catalog no.	929002
Number of assays	12 x 100
QIAxcel DNA High Resolution Cartridge (with smart key)	1
QX Separation Buffer*	40 ml
QX Wash Buffer*	40 ml
QX Mineral Oil	50 ml
QX DNA Dilution Buffer	15 ml
QX Intensity Calibration Marker	1 ml
QX 0.2 ml 12-Tube Strips	2
QX Colored 0.2 ml 12-Tube Strips	2
Handbook	1

* Contains sodium azide as a preservative.

QIAxcel DNA Screening Kit	(2400)
Catalog no.	929004
Number of assays	12 x 200
QIAxcel DNA Screening Cartridge (with smart key)	1
QX Separation Buffer*	40 ml
QX Wash Buffer*	40 ml
QX Mineral Oil	50 ml
QX DNA Dilution Buffer	15 ml
QX Intensity Calibration Marker	1 ml
QX 0.2 ml 12-Tube Strips	2
QX Colored 0.2 ml 12-Tube Strips	2
Handbook	1

* Contains sodium azide as a preservative.

QIAxcel DNA Fast Analysis Kit	(3000)
Catalog no.	929008
Number of assays	12 x 250
QIAxcel DNA Fast Analysis Cartridge (with smart key)	1
QX FA Separation Buffer*	40 ml
QX Wash Buffer	40 ml
QX Mineral Oil	50 ml
QX Intensity Calibration Marker	1 ml
QX 0.2 ml 12-Tube Strips	2
QX Colored 0.2 ml 12-Tube Strips	2
QX DNA Size Marker 50 bp – 1.5 kb	100 μ l
QX Alignment Marker 15 bp/3 kb	1.5 ml
Handbook	1

The QIAxcel DNA Fast Analysis Kit is not suited for concentration determination.

* Contains sodium azide as a preservative.

Unpacking the QIAxcel Gel Cartridge

For optimal performance, store the QIAxcel gel cartridge at 2–8°C until the first use. Prior to use, the QIAxcel gel cartridge should be placed into the QX Cartridge Stand and protected with the cover whenever exposed to light, or stored latched in the instrument in the “Park Position” with buffer in the buffer tray, and allowed to stand for at least 20 minutes (for more information, see “Preparing the QIAxcel gel cartridge and buffer tray”, page 12).

Storage

All components of the QIAxcel DNA High Resolution Kit, and the QIAxcel DNA Screening Kit, except for the gel cartridge and the QX Intensity Calibration Marker, can be stored dry at room temperature (15–25°C). The QIAxcel gel cartridge and the marker should be stored at 2–8°C upon arrival.

All components of the QIAxcel DNA Fast Analysis Kit, except for the gel cartridge and the markers, can be stored dry at room temperature. The QIAxcel gel cartridge and the markers should be stored at 2–8°C upon arrival.

Store QIAxcel DNA gel cartridges at 2–8°C until the first use. If the QIAxcel gel cartridge is not being used on a daily basis, close the purge port with the purge port seal, return the QIAxcel DNA gel cartridge to the blister package, inserting the capillary tips into the soft gel, and store at 2–8°C in an upright position (see orientation label on blister package).

Note: Storing the QIAxcel DNA gel cartridge below 2°C can severely damage the cartridge.

Prior to use, the QIAxcel DNA gel cartridge should be placed into the QX Cartridge Stand and protected with the cover whenever exposed to light, or stored latched in the instrument in the “Park Position” with buffer in the buffer tray, and allowed to stand for at least 20 minutes.

If the QIAxcel DNA gel cartridge is being used on a daily basis, store the cartridge latched in the QIAxcel instrument in the “Park Position” (refer to Section 5.3 of the *QIAxcel User Manual* or the *QIAxcel Advanced User Manual*).

Note: The QIAxcel instrument must be left on if the cartridge is stored in the “Park Position” and the cartridge must be latched. Do not switch the QIAxcel instrument off.

If more than one QIAxcel DNA gel cartridge is being used on a daily basis, store the second cartridge in the QX Cartridge Stand in the dark or protected with the cover. Make sure that the cartridge stand reservoir is filled with wash buffer and covered with mineral oil (refer to Section 5.1 of the *QIAxcel User Manual* or Section 5.2.5 of the *QIAxcel Advanced User Manual*). Alternatively, close the purge port with the purge port seal, return the QIAxcel DNA gel cartridge to the blister package, inserting the capillary tips into the soft gel, and store at 2–8°C in an upright position (see orientation label on blister package).

The QIAxcel DNA gel cartridge can be stored in this manner until the expiration date indicated on the kit label.

Intended Use

QIAxcel DNA Kits are intended for molecular biology applications. These products are 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.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAxcel DNA High Resolution Kit, QIAxcel DNA Screening Kit, and QIAxcel DNA Fast Analysis Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

QIAxcel instruments, in combination with the QIAxcel DNA High Resolution Kit, the QIAxcel DNA Screening Kit, and the QIAxcel DNA Fast Analysis Kit, provide fully automated separation of DNA fragments according to size, with processing of up to 96 samples per run.

QIAxcel technology, based on capillary electrophoresis using QIAxcel gel cartridges, provides unmatched resolution, speed, and throughput. QIAxcel gel cartridges are reusable, allowing multiple runs of 12 samples to be performed (up to 100 runs with the QIAxcel DNA High Resolution Kit, 200 runs with the QIAxcel DNA Screening Kit, and 250 runs with the QIAxcel DNA Fast Analysis Kit). QIAxcel instruments are preinstalled with methods suitable for most applications. In addition, customized methods can also be created — contact QIAGEN Technical Services for more details.

QIAxcel ScreenGel® software supplied with the QIAxcel Advanced instrument provides both electropherogram and gel images of nucleic acid separation.

Kits for separation and quantification of RNA and protein are also available (see ordering information, page 51).

Principle and procedure

The QIAxcel Advanced system uses capillary gel electrophoresis to enable fast separation of nucleic acids based on size. Unlike traditional agarose gel electrophoresis, separation is performed in a capillary of a precast gel cartridge. Each sample is automatically loaded into an individual capillary (according to voltage and time parameters) and voltage is applied. The negatively charged nucleic acid molecules migrate through the capillary to the positively charged end (Figure 1, page 10). As with agarose gel electrophoresis, low-molecular-weight molecules migrate faster than high-molecular-weight molecules. As the molecules migrate through the capillary, they pass a detector that detects and measures the fluorescent signal. A photomultiplier detector converts the emission signal into electronic data, which are then transferred to the computer for further processing using QIAxcel ScreenGel software or BioCalculator software. After processing, the data are displayed as an electropherogram or gel image.

The QIAxcel Advanced system offers a number of advantages over traditional agarose gel electrophoresis, including:

- Higher detection sensitivity
- Less sample wastage (minimal sample input volumes)
- Improved fragment resolution
- Fast analysis of up to 96 samples
- Automated loading and analysis

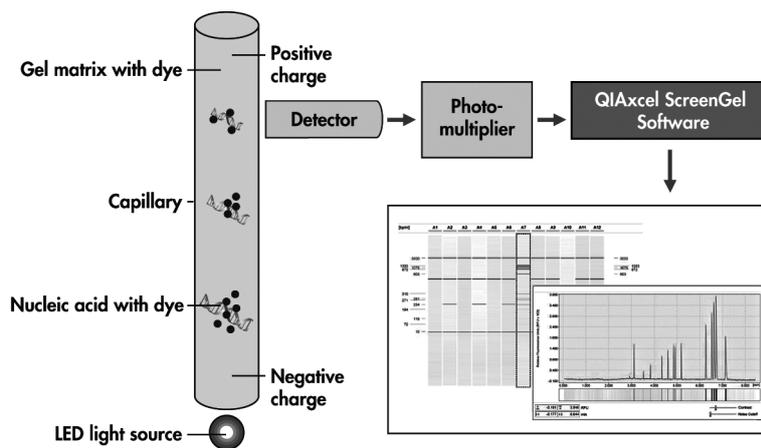


Figure 1. Sample separation process using the QIAxcel Advanced system. Nucleic acid molecules are separated according to size by applying a voltage to a gel-filled capillary. A photomultiplier detector in the instrument detects the staining dye molecules as they migrate towards the positively charged end of the capillary. The data are converted to an electropherogram and a gel image by the QIAxcel ScreenGel software or the BioCalculator software (not shown).

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 safety data sheets (SDSs), available from the product supplier.

For all users

- Pipets and pipet tips
- 12-tube strips (e.g., QX 0.2 ml 12-Tube Strip, cat. no. 929703) or 96-well plates
- Centrifuge with rotor suitable for 0.2 ml strips or 96-well plates, such as the Centrifuge 4-16 or Centrifuge 4-16K (for ordering information, see www.qiagen.com)
- QX Alignment Marker (see “Marker selection”, page 18)*†
- QX DNA Size Marker (see “Marker selection”, page 18)*†
- **Optional:** QX DNA Dilution Buffer (cat. no. 929601) may be required to fill empty wells

For QIAxcel users

- QIAxcel instrument and BioCalculator Software or QIAxcel ScreenGel software version 1.1.0 or higher (cat. no. 9021163)

For QIAxcel Advanced users

- QIAxcel Advanced instrument (cat. no. 9001941) and QIAxcel ScreenGel software version 1.1.0 or higher (license included with cat. no. 9001941)

* For recommended combinations of QX Alignment Marker and QX DNA Size Marker, refer to Table 2, page 20.

† QX Alignment Marker 15 bp/3 kb and QX DNA Size Marker 50 bp – 1.5 kb are supplied with the QIAxcel DNA Fast Analysis Kit.

Important Notes

Preparing the QIAxcel gel cartridge and buffer tray

This procedure describes how to prepare the QIAxcel DNA High Resolution Cartridge, QIAxcel DNA Screening Cartridge, or QIAxcel DNA Fast Analysis Cartridge and buffer tray prior to DNA analysis.

Important points before starting

- The volume of buffer supplied is sufficient for a defined number of sample runs (see Table 1). If required, additional buffers can be purchased separately (see ordering information, page 53).

Table 1. Number of runs possible using the supplied buffer volumes

Kit	Number of runs	Samples per run
QIAxcel DNA High Resolution Kit	100	12
QIAxcel DNA Screening Kit	200	12
QIAxcel DNA Fast Analysis Kit	250	12

- The 0.2 ml 12-tube strips containing QX Alignment Marker and QX Intensity Calibration Marker (if required) should fit loosely in the MARKER1 and MARKER2 positions (see steps 12 and 19).
- QX Alignment Markers should be replaced every 50 runs or 3 days, whichever comes first. Additional markers and buffers may need to be purchased (see ordering information, page 53).
- When not in use, the 12-tube strip containing QX Alignment Marker should be stored at -20°C .
- For optimal performance, store the QIAxcel gel cartridge at $2-8^{\circ}\text{C}$ until required for use. Prior to use, the QIAxcel gel cartridge should be placed into the QX Cartridge Stand and protected with the cover, or stored latched in the instrument in the "Park Position" with buffer in the buffer tray, and allowed to stand for at least 20 minutes.

Things to do before starting

- If prepared in advance, the 12-tube strip containing QX Alignment Marker should be equilibrated to room temperature (20–25°C) and centrifuged briefly before use.
- If the QIAxcel DNA gel cartridge is being used for the first time, intensity calibration should be performed (refer to “Intensity calibration”, page 16, and Section 5.4 of the *QIAxcel User Manual* or Section 6.5.1 of the *QIAxcel Advanced User Manual*). This is not necessary if the QIAxcel DNA gel cartridge has already been calibrated, unless it is being used on a different QIAxcel or QIAxcel Advanced instrument or a different computer is used to operate the instrument. If a different computer is being used to operate the QIAxcel or QIAxcel Advanced instrument, the calibration log file must be transferred to the new computer so that calibration does not need to be performed again.

Unpacking and preparing the QIAxcel gel cartridge

For optimal performance, store the QIAxcel DNA gel cartridge at 2–8°C until required for use. Prior to use, the QIAxcel DNA gel cartridge should be placed into the QX Cartridge Stand protected with the cover, or stored latched in the instrument in the “Park Position” with buffer in the buffer tray, and allowed to stand for at least 20 minutes.

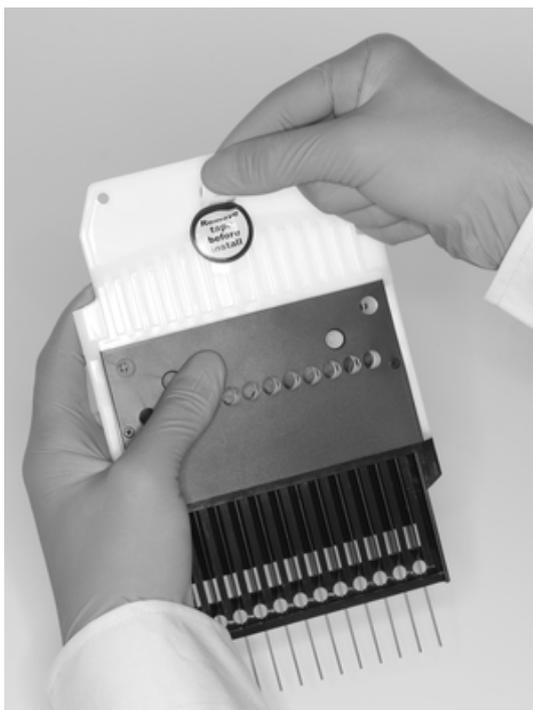
1. Remove all buffer bottles from the kit box.
2. Add 10 ml QX Wash Buffer to both reservoirs of the QX Cartridge Stand (provided with QIAxcel instruments) and cover with 2 ml mineral oil (supplied).
3. Remove the QIAxcel DNA gel cartridge from its packaging and carefully wipe off any soft gel debris from the capillary tips using a soft tissue.
4. Remove the purge cap seal from the back of the QIAxcel DNA gel cartridge and place the gel cartridge in the QX Cartridge Stand. Retain the purge port seal in case you need to store the QIAxcel DNA gel cartridge.

Note: Use a soft tissue to wipe off any gel that may have leaked from the purge port.

Note: Ensure that the capillary tips are submerged in QX Wash Buffer.

5. Incubate new cartridges in the QX Cartridge Stand for at least 20 minutes prior to use.

Note: Once used, the QIAxcel DNA gel cartridge must be stored in a vertical orientation. For more information, see “Storage”, page 6.



Preparing the QIAxcel gel cartridge.

Preparing the buffer tray

1. Allow all reagents to equilibrate to room temperature (15–25°C) before use.
2. Wash the buffer tray with hot water and rinse thoroughly with deionized water.
3. Fill the WP and WI positions of the buffer tray with 8 ml QX Wash Buffer.
4. Fill the BUFFER position of the buffer tray with 18 ml QX Separation Buffer or, if using a QIAxcel DNA Fast Analysis Cartridge, 18 ml QX FA Separation Buffer.
5. Carefully add mineral oil to cover all 3 positions to prevent evaporation: add 2 ml mineral oil to positions WP and WI and add 4 ml mineral oil to position BUFFER.
6. Insert the buffer tray into the buffer tray holder so that the slots for the 12-tube strips face the front of the instrument.

Preparing QX Alignment Marker

1. Load 15 μ l QX Alignment Marker into each tube of a QX 0.2 ml 12-Tube Strip.
2. Add 1 drop of mineral oil to each tube, and place the strip into the MARKER1 position of the buffer tray.

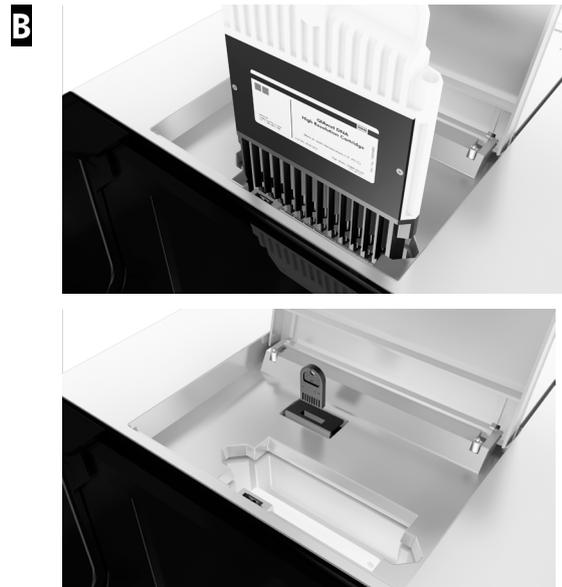


Preparing the buffer tray and inserting the buffer tray into the buffer tray holder.

Installing a QIAxcel DNA gel cartridge and smart key

1. Remove the QIAxcel DNA gel cartridge from the QX Cartridge Stand.
2. Open the cartridge door and place the QIAxcel DNA gel cartridge into the QIAxcel or QIAxcel Advanced instrument. The cartridge description label should face the front and the purge port should face the back of the instrument.
3. Insert the smart key into the smart key socket. The smart key can be inserted in either direction.
4. Close the cartridge door.
5. The cartridge identifier, number of runs remaining, and cartridge type will be displayed automatically in the software when the cartridge smart key is inserted.

Note: The system will not recognize the cartridge and will not operate if the smart key is not inserted.



Installing the QIAxcel gel cartridge and smart key in the **A** QIAxcel and **B** QIAxcel Advanced instruments.

Intensity calibration

Every QIAxcel DNA gel cartridge requires intensity calibration prior to sample analysis. The intensities of each capillary are normalized and a factor is applied for every subsequent run. This corrects for natural intensity reading variations between each capillary in the cartridge.

Intensity calibration using QIAxcel ScreenGel Software

The data for each cartridge intensity calibration are stored in a file named according to the cartridge and instrument identifiers (<cartridge-id>_<instrument-id>.xcc). This file is saved either in the default directory **C:\Documents and Settings\All Users\Application Data\QIAGEN\QIAxcel\ScreenGel\Data\CartridgeCalibrationData** or in a customized directory.

If, for any reason, a different computer is used to the one on which the calibration file is saved, the calibration file should be transferred to the new computer. Otherwise, recalibration of the cartridge is required. Similarly, if the QIAxcel gel cartridge is used on a different QIAxcel instrument to the one it was calibrated on, another intensity calibration should be performed.

Intensity calibration of the cartridge takes about 16 minutes.

1. Load 15 μ l QX Intensity Calibration Marker into each tube of a QX Colored 0.2 ml 12-Tube Strip. Add a drop of mineral oil, and insert the strip into the MARKER2 position of the buffer tray.
2. Launch the calibration run by clicking the "Start calibration" button in the "Calibration" screen of the "Service" environment.
3. Once the calibration is complete, the calibration results are displayed next to the gel image or the electropherogram view. The result table shows the area, calibration factor, and the result ("Pass" or "Fail") for each channel.
Note: A successfully calibrated cartridge should have a normalized area calibrated range between 0.004–0.006 or, if using a QIAxcel DNA Fast Analysis Cartridge, between 0.0035–0.0065.
4. If one or more channels show no signals in the first run, refer to Appendix D, page 44.
5. If one or more channels show high background, refer to Section 8 of the *QIAxcel User Manual* or the *QIAxcel Advanced User Manual*.
6. If calibration fails more than twice, call QIAGEN Technical Services.

Recalibration using QIAxcel ScreenGel Software

To recalibrate a cartridge, repeat the procedure described in "Intensity calibration using QIAxcel ScreenGel Software". The calibration results of the previous calibration procedure are discarded when recalibrating a cartridge.

Note: It is possible to calibrate a cartridge for which no calibration runs remain. In this case, 3 of the remaining regular runs are used instead of 1 calibration run.

Intensity calibration using BioCalculator Software

The data (individual calibration data files) for each cartridge intensity calibration are stored in the **CALdata** folder. This folder is saved in the BioCalculator root directory: **C:\Program Files\QIAxcel BioCalculator**.

A **Calibration2.log** file (cartridge calibration information) is saved automatically in the BioCalculator root directory: **C:\Program Files\QIAxcel BioCalculator**.

If, for any reason, a different computer is used to the one on which the **Calibration2.log** file is saved, the **Calibration2.log** file should be transferred to the new computer. Otherwise, recalibration of the cartridge is required. Similarly, if the QIAxcel gel cartridge is used on a different QIAxcel instrument to the one it was calibrated on, another intensity calibration should be performed.

Intensity calibration of the cartridge takes about 16 minutes.

1. Load 15 μ l QX Intensity Calibration Marker into each tube of a QX Colored 0.2 ml 12-Tube Strip, make sure no air bubbles are trapped in the solution, and place it into the MARKER2 position of the buffer tray.
2. Launch the calibration wizard by selecting "File" then "Intensity Calibration" in the "Instrument Control" window.
3. Click "Start" to begin the cartridge intensity calibration.
4. When calibration is complete, the "Calibration Verification" dialog box will open. This will show either "Pass" or "Fail" for each channel.

Note: A successfully calibrated cartridge should have a normalized area calibrated range between 0.004–0.006 or, if using a QIAxcel DNA Fast Analysis Cartridge, between 0.0035–0.0065.

5. If one or more channels fail, repeat the calibration process using a new strip of QX Intensity Calibration Marker (see page 18).
6. If one or more channels show no signals in the first run, refer to Appendix C, page 38.
7. If one or more channels show high background, refer to Section 8 of the *QIAxcel User Manual* or the *QIAxcel Advanced User Manual*.
8. If calibration fails more than twice, call QIAGEN Technical Services.

Recalibration using BioCalculator Software

Note: Use a new strip of QX Intensity Calibration Marker for each recalibration run. Re-using the intensity calibration marker may affect the calibration data and can cause them to be out of range.

1. Load 15 μ l QX Intensity Calibration Marker into each tube of a new QX Color 0.2 ml 12-Tube Strip. Make sure there are no air bubbles, and place the strip into the MARKER2 position of the buffer tray.
2. Launch the calibration wizard by selecting "File" then "Intensity Calibration" in the "Instrument Control" window.
3. Click "Recalibrate" and then "Start" to repeat the calibration routine.

Sample preparation recommendations

The minimum sample volume required for analysis is 10 μ l. Less than 0.1 μ l of the sample will be injected into the QIAxcel gel cartridge for analysis. The remaining DNA can be kept for reanalysis or use in downstream applications such as sequencing or cloning. We do not recommend using the remaining DNA in amplification-based applications such as real-time PCR or nested PCR.

Marker selection

Alignment marker

The QIAxcel DNA Fast Analysis Kit contains QX Alignment Marker 15 bp/3 kb (cat. no. 929522) which should be used in combination with the corresponding methods. For optimal DNA fragment size determination using the QIAxcel DNA High Resolution Kit or the QIAxcel DNA Screening Kit, select a QX Size Marker with a fragment size close to the size of your samples (see ordering information, page 51). Ensure that the fragment of interest is smaller than the largest peak of the chosen size marker. A product finder is available at www.qiagen.com/QXmarker to help with selection. For example, if analyzing a sample containing DNA fragments that are 100–400 bp in size, QX Alignment Marker 15 bp/600 bp (cat. no. 929530) should be used. For general applications using the QIAxcel DNA High Resolution Kit or the QIAxcel DNA Screening Kit, QX Alignment Marker 15 bp/5 kb (cat. no. 929524) is suitable.

Alignment markers are injected from the MARKER1 position of the buffer tray and co-migrate with the DNA samples for analysis.

DNA size marker

QIAxcel ScreenGel software or BioCalculator software calculates the DNA fragment size based on the fragment migration time in comparison to a reference QX DNA Size Marker (see ordering information, page 51). The DNA size (bp) is calculated using a point-to-point calculation using 2 DNA size marker fragments. To enable accurate size measurements, the size of the DNA fragments to be analyzed must fall within the smallest and largest fragment sizes of the QX DNA Size Marker.

The QIAxcel DNA Fast Analysis Kit contains the ready-to-use QX DNA Size Marker 50 bp – 1.5 kb. This marker should be used undiluted and is suitable for most applications of the QIAxcel DNA Fast Analysis Kit.

For optimal DNA size determination using the QIAxcel DNA High Resolution Kit or the QIAxcel DNA Screening Kit, select a QX DNA Size Marker with fragments closest to the size of your DNA sample. For optimal concentration determination, we recommend diluting the QX DNA Size Marker in the same buffer that was used for the DNA samples. For undiluted DNA samples, we recommend diluting the 100 ng/ μ l QX DNA Size Marker in 1x PCR buffer or 1x restriction digestion buffer to the following final concentration:

- 10 ng/ μ l for L methods
- 30 ng/ μ l for M methods
- 50 ng/ μ l for H methods

For samples diluted in QX DNA Dilution Buffer, we recommend also diluting the DNA size marker to a final concentration of 5 ng/ μ l in QX DNA Dilution Buffer.

Table 2 provides recommendations for combining QX DNA Size Markers and QX Alignment Markers.

Table 2. Recommended QX DNA Size Marker and QX Alignment Marker combinations

Size marker	Cat. no.	Alignment marker	Cat. no.
QX DNA Size Marker pUC18/ <i>Hae</i> III (50 μ l)	929550	QX Alignment Marker 15 bp/1 kb (1.5 ml)	929521
QX DNA Size Marker FX174/ <i>Hae</i> III (50 μ l)	929551	QX Alignment Marker 15 bp/3 kb (1.5 ml)	929522
QX DNA Size Marker 25 bp – 500 bp (50 μ l)	929560	QX Alignment Marker 15 bp/600 bp (1.5 ml)	929530
QX DNA Size Marker 100 bp – 2.5 kb (50 μ l)	929559	QX Alignment Marker 15 bp/3 kb (1.5 ml)	929522
QX DNA Size Marker 50–800 bp (50 μ l) v2.0	929561	QX Alignment Marker 15 bp/1 kb (1.5 ml)	929521
QX DNA Size Marker 250 bp – 4 kb (50 μ l) v2.0	929562	QX Alignment Marker 50 bp/5 kb (1.5 ml)	929529
QX DNA Size Marker 250 bp – 8 kb (50 μ l) v2.0	929563	QX Alignment Marker 15 bp/10 kb (1.5 ml)	929523

Method selection

A number of preinstalled methods are available for QIAxcel DNA gel cartridges. To select the appropriate method for the samples being analyzed, refer to Appendix C, page 38, or Section 5.5 of the *QIAxcel User Manual* or Section 6.3.3 of the *QIAxcel Advanced User Manual*.

Protocol: Determination of DNA Fragment Sizes using QIAxcel ScreenGel Software

Important points before starting

- Before beginning the procedure, read “Important Notes” beginning on page 12.
- For optimal results, DNA should be in a solution of approximately pH 6–9 and should not have an ionic content greater than that of a typical PCR buffer.
- Determine the optimal QIAxcel method for sample analysis (refer to Appendix C, page 38, or Section 5.5 of the *QIAxcel User Manual* or Section 6.3.3 of the *QIAxcel Advanced User Manual* for more details).

Things to do before starting

- Ensure samples have been prepared according to the instructions in “Sample preparation recommendations”, page 18.
- If the QIAxcel gel cartridge was stored at 2–8°C, place it into the QX Cartridge Stand or the instrument, latched in the “Park Position” with buffer in the buffer tray, and allow to stand for at least 20 minutes prior to use.
- Ensure that the QIAxcel gel cartridge is correctly set up and all reagents have been prepared according to the instructions in “Preparing the QIAxcel gel cartridge and buffer tray”, page 12.

Optional: Create a DNA reference marker table before running samples (see Appendix A, page 29, for more details). The table can be prepared after the sample run, if required.

Note: The steps in this protocol are based on the default process profile while logged in as a routine user.

- 1. Switch on the QIAxcel instrument.**
- 2. Switch on the computer, launch the QIAxcel ScreenGel software, and log in in “DNA mode” as a routine user.**
- 3. Install the QIAxcel DNA gel cartridge.**

Refer to Section 5.2.3 of the *QIAxcel User Manual* or the *QIAxcel Advanced User Manual* for more details.

Note: If being used for the first time, the QIAxcel gel cartridge will require calibration (see “Preparing the QIAxcel gel cartridge and buffer tray” page 12).

4. Load the buffer tray containing the QX Alignment Marker into the buffer tray holder.

Refer to Section 5.2.2 of the *QIAxcel User Manual* or the *QIAxcel Advanced User Manual* for more details.

Note: QX Alignment Markers should be replaced every 50 runs or 3 days, whichever comes first. When not in use, the 12-tube strip containing QX Alignment Marker should be stored at -20°C .

5. Load the sample strips or load a 96-well plate containing samples onto the sample tray holder.

Note: The cartridge door and sample door of the QIAxcel instrument must remain closed during operation of the instrument. Opening the cartridge door or sample door during operation will cause the system to stop any action it is currently performing.

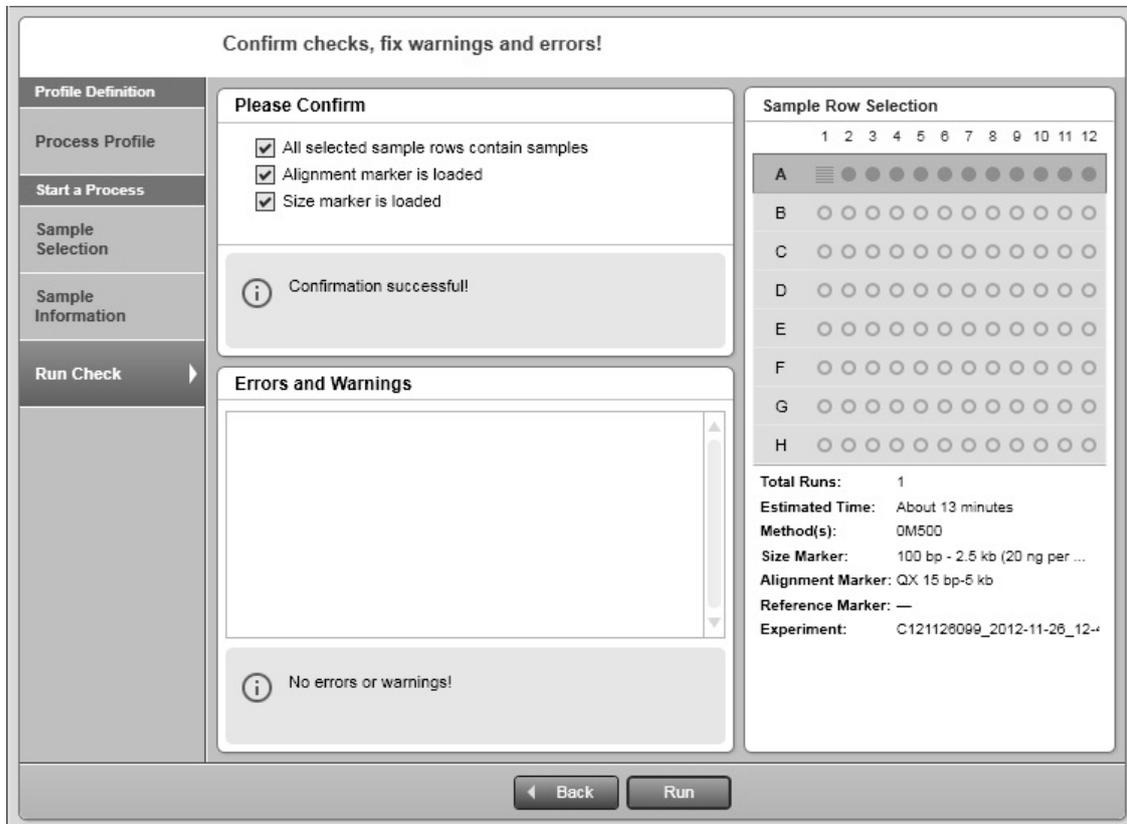
6. Select a process profile from the drop-down list.

Note: Process profiles provide preset analysis and report parameters for samples. Default process files or user-created files can be used. See Appendix A, page 29 or Section 6.3 of the *QIAxcel Advanced User Manual* for a description of how to create process profiles.

7. Click “Next” to open the “Sample Selection” tab.

The following information can be modified in this tab: size and alignment marker selection and position, lot number information, and the automatically generated plate identifier.

8. Click "Next" to open the "Sample Information" tab.
Information about the sample can be entered.
9. Click "Next" to open the "Run Check" tab, and confirm that samples and markers have been loaded correctly.
10. Click "Run" to start the run.



Note: A report is automatically generated according to the settings in the selected process profile.

Note: Changes to the analysis settings can be performed by an advanced user.

Protocol: Determination of DNA Fragment Sizes using BioCalculator Software

Important points before starting

- Before beginning the procedure, read “Important Notes” beginning on page 12.
- For optimal results, DNA should be in a solution of approximately pH 6–9 and should not have an ionic content greater than that of a typical PCR buffer.
- Determine the optimal QIAxcel method for sample analysis (refer to Appendix C, page 38, or Section 5.5 of the *QIAxcel User Manual* for more details).

Things to do before starting

- Ensure samples have been prepared according to the instructions in “Sample preparation recommendations”, page 18.
- If the QIAxcel gel cartridge was stored at 2–8°C, place it into the QX Cartridge Stand or the instrument latched in the “Park Position” with buffer in the buffer tray, and allow to stand for at least 20 minutes prior to use.
- Ensure that the QIAxcel gel cartridge is correctly set up and all reagents have been prepared according to the instructions in “Preparing the QIAxcel gel cartridge and buffer tray”, page 12.
- **Optional:** Create a DNA reference marker table before running samples (see Appendix A, page 29, for more details). The table can be prepared after the sample run, if required.

Procedure

1. **Switch on the QIAxcel instrument.**
2. **Switch on the computer and launch the BioCalculator software.**
3. **Install the QIAxcel gel cartridge.**

Refer to Section 5.2.3 of the *QIAxcel User Manual* for more details.

4. Load the buffer tray containing the QX Alignment Marker into the buffer tray holder.

Refer to Section 5.2.2 of the *QIAxcel User Manual* for more details.

Note: If being used for the first time, the QIAxcel gel cartridge will require calibration (see “Preparing the QIAxcel gel cartridge and buffer tray” page 12).

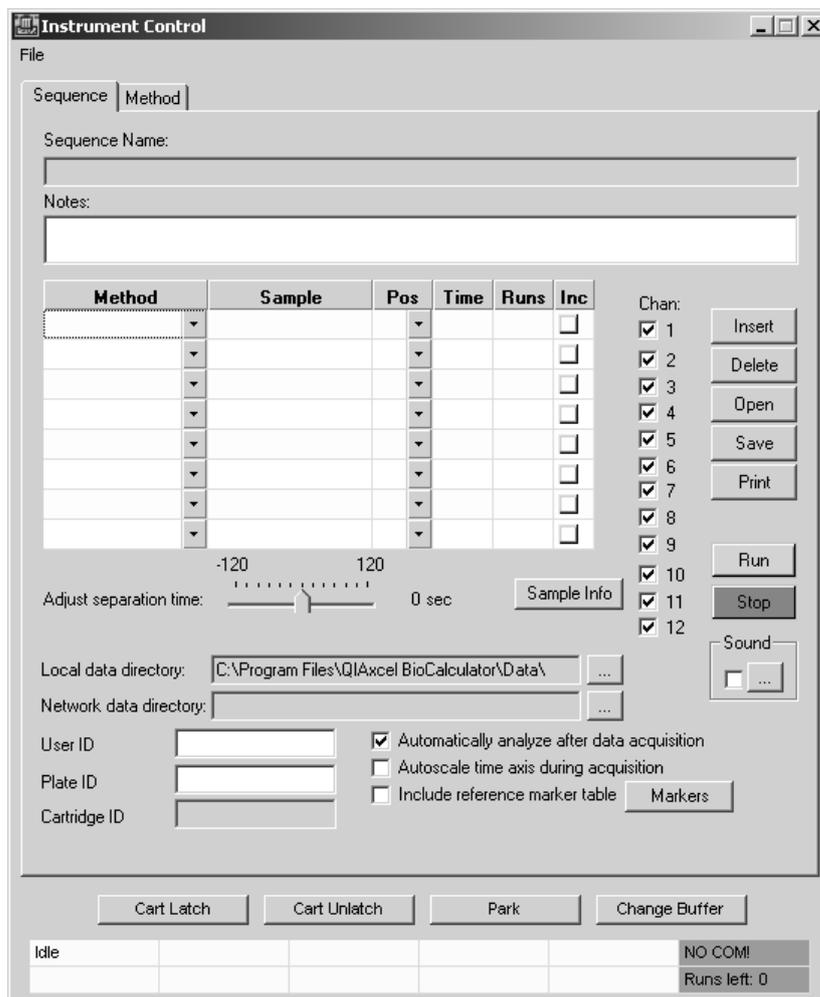
Note: QX Alignment Markers should be replaced every 50 runs or 3 days, whichever comes first. When not in use, the 12-tube strip containing QX Alignment Marker should be stored at -20°C .

5. Load the sample strips or load a 96-well plate containing samples onto the sample tray holder.

Note: The cartridge door and sample door of the QIAxcel system must remain closed during operation of the instrument. Opening the cartridge door or sample door during operation will cause the system to stop any action it is currently performing.

6. Select the appropriate method in the “Instrument Control” window.

Refer to Appendix C, page 38, or Section 5.5 of the *QIAxcel User Manual* for more information about preinstalled methods.



7. **Enter the sample name, position, and number of runs in the corresponding fields of the “Instrument Control” window.**
8. **Optional: Enter the sample injection time (minimum: 1 s; maximum: 60 s or, when using a QIAxcel DNA Fast Analysis Cartridge, 40 s) into the “Time” field. We recommend using a minimum of 5 s and a maximum of 15 s.**

If the “Time” field is left blank, the QIAxcel system uses the default settings for the selected method.

9. **To perform multiple analyses of the same row, enter the number of repeats in the “Runs” field and leave the increments box (“Inc”) unchecked.**

To process all rows of a 96-well plate using the same method, check the increments box (“Inc”) and enter 8 in the “Runs” field.

10. **Select the data directory where the run should be saved.**

Note: Subfolders will be created in the data directory if information is entered into the “User ID” and “Plate ID” fields.

Optional: If connected to a network, in addition to a local data directory, you are able to select a network directory in which the run data will be saved.

11. **Recommended: Click the “Sample Info” button to enter sample information for each well.**

Alternatively, sample information in a spreadsheet can be imported in *.csv (comma-separated value) file format.

12. **Make sure to check the separation channels to be used (i.e., if running less than the maximum number of samples, check only the channels that contain samples).**

Note: Unused wells should contain QX DNA Dilution Buffer to prevent damage to the channel.

13. **Optional: Check the “Automatically analyze after data acquisition” box.**

When this option is checked, the data will be automatically analyzed according to the parameter settings after the raw data has been acquired.

14. **Optional: Check the “Include reference marker table” box. Then click the “Marker” button and open the desired DNA reference marker table.**

When this option is checked, DNA size determination is performed automatically. Ensure that the same method used to create the DNA reference marker table is used for sample analysis. See Appendix A, page 29, for more information.

15. Check the status of the QIAxcel system in the status panel.

Make sure that the cartridge door and sample door are closed.

Note: The status panel is at the bottom of the “Instrument Control” window and displays information about the status of the QIAxcel system (refer to Section 5.3 of the *QIAxcel User Manual* for more details).

16. Click “Run” to start sample processing.

At the start of the run, a window displaying electropherogram and gel images opens.

Troubleshooting Guide

The *QIAxcel User Manual* and the *QIAxcel Advanced User Manual* contain a troubleshooting guide, which may be helpful in solving any problems that may arise. In addition, extensive user information is also provided in the “Help” menu of QIAxcel ScreenGel software and BioCalculator software. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. 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 sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

Appendix A: Creating a Process Profile in QIAxcel ScreenGel Software

Note: Only users with the assigned role “Advanced User” can create a new process profile.

To create a new process profile, proceed as follows:

A1. Open the “Process” environment.

If not open, switch to the “Process” environment by clicking the “Process” environment icon. Select the “Process Profile” screen.

The screenshot shows the 'Process Setup' window with the following details:

- Process Setup** (Title Bar)
- Set the process profile!** (Section Header)
- Profile Definition** (Tab)
- Process Profile** (Selected Tab)
- Run Parameters** (Tab)
- Analysis** (Tab)
- Marker** (Tab)
- Peak Calling** (Tab)
- Report/Export** (Tab)
- Start a Process** (Section Header)
- Sample Selection** (Tab)
- Sample Information** (Tab)
- Run Check** (Tab)

Settings in the 'Process Profile' tab:

- Mode** (Section Header)
- Cartridge Type**: DNA Screening (Dropdown menu)
- Process Profile**: *NewProcessProfile (Dropdown menu)
- Profile** (Section Header)
- Cartridge Type**: DNA Screening (Text field)
- Included Steps**: Run, Analysis, Peak Calling, Report
- Experiment Directory**: ata\QIAGEN\QIAxcel\ScreenGel\Data\Experiment\DNA (Text field with browse button)
- Allow Directory Selection
- Notes** (Text area)

Buttons at the bottom:

- Save process profile as ...** (Highlighted)
- Run**

Main steps for creation of a new process profile.

Note: If the last process only just finished, click “Back to Wizard” at the bottom right.

A2. Select cartridge type.

Select the cartridge type that is to be used for the new process profile.

Note: Each process profile is related to a certain cartridge type. Therefore, the system can ensure that a process (i.e., data acquisition) can be started only if a cartridge with correct cartridge type is inserted.

Note: If the instrument is connected, the system automatically detects the inserted cartridge and the cartridge type cannot be changed. If you want to create the process profile for another cartridge type, remove the cartridge, or at least the cartridge key, or disconnect from the instrument.

A3. Select a process profile.

Use the "Process Profile" drop-down list to select a process profile. The selected profile serves as a template for the creation of the new profile.

Note: Select "NewProcessProfile" to create a process profile from scratch. The system will show "*NewProcessProfile" after selection. This can only be carried out by users with the assigned role "Advanced User".

A4. Set the process profile options according to your needs.

See Section 6.3.3 of the *QIAxcel Advanced User Manual* for detailed information.

A5. Save the modified process profile under a new name.

Click the "Save Process Profile as ..." button below process setup and enter a new unique profile name in the "Save Profile" dialog box that appears.

Note: This can only be carried out by users with the assigned user role "Advanced User".

Note: If there are process profile screens containing incomplete or inconsistent data highlighted in yellow, the "Save process profile as..." button is disabled. Select the marked process profile screens and correct the data. If all data are correct, the "Save process profile as..." button becomes enabled and the process profile can be saved.

Appendix B: Data Analysis

Aligning the gel image with BioCalculator software

After the run, a window containing the gel image is displayed. This is referred to as the "Folder View" (see Figure 2). Additional windows display data for each individual channel. Data from an individual channel can be opened by double-clicking the appropriate channel.

Generated data are automatically aligned by the BioCalculator software if the "Automatically analyze after data acquisition" box is checked; however, manual

alignment is possible (see procedure below). The alignment compensates for any slight variations in migration speed between the different capillaries and aligns the first and last peak (band) across all 12 channels.

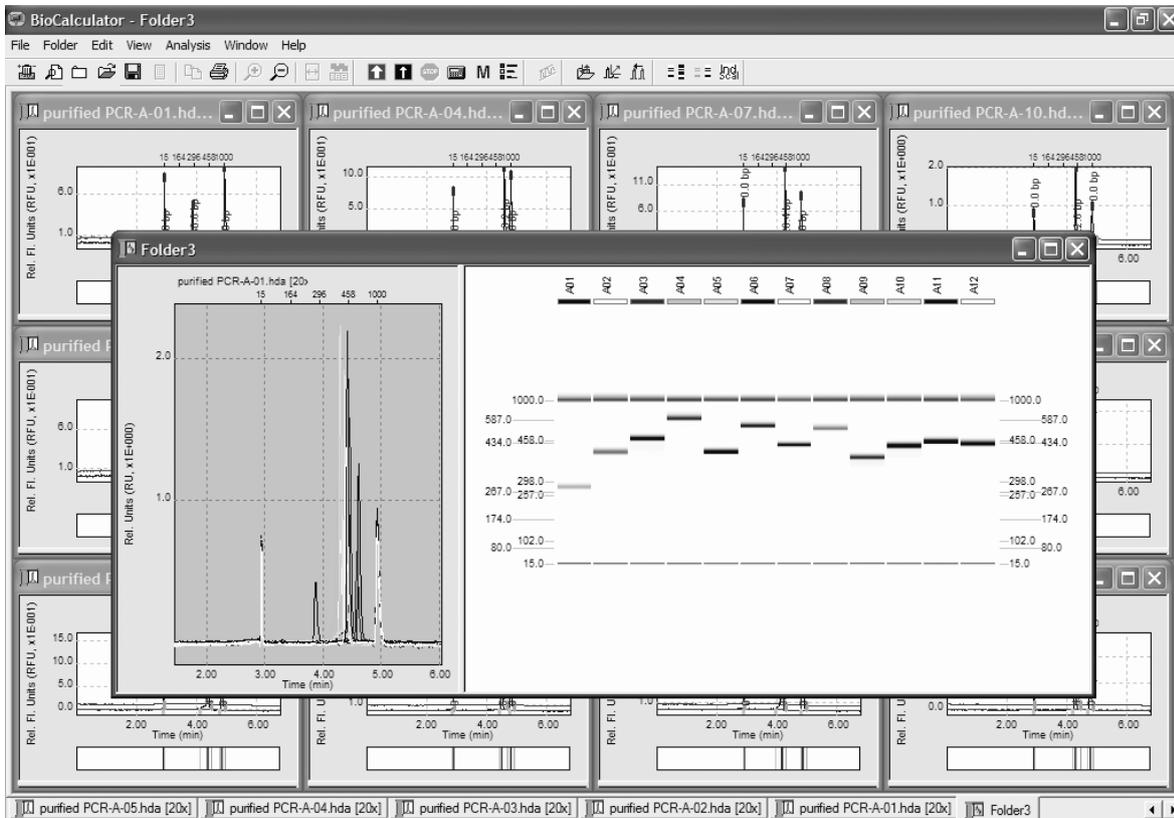
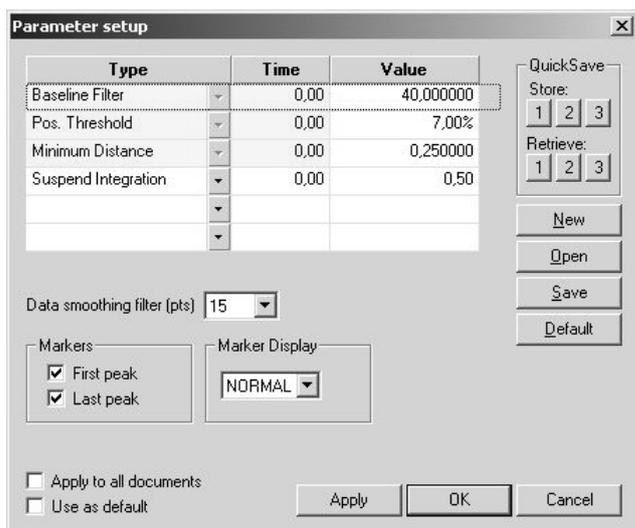


Figure 2. The “Folder View”.

Manual alignment procedure

- B1. Click “Analysis” and then “Parameters” in the menu bar to display the “Parameter setup” dialog box.**
- B2. In the “Data Smoothing filter (pts)” drop-down list, select 15 when using the QIAxcel DNA High Resolution Kit or the QIAxcel DNA Screening Kit or OFF when using the QIAxcel DNA Fast Analysis Kit.**
- B3. In the “Markers” panel, check “First peak” and “Last Peak”.**
- B4. Check “Apply to all documents” and click “OK”.**



B5. Select “Analysis” and then “Run” in the menu bar. If the peaks or bands are not aligned, refer to Section 8 in the *QIAxcel User Manual* for further guidance.

Creating a DNA size marker reference table

The DNA size marker reference table allows accurate size determination of sample DNA fragment sizes.

For the QIAxcel DNA Fast Analysis Kit, a DNA size marker run should be performed every working day, since variations in separation temperature of the cartridge and buffer can introduce variations in DNA size determination. Whenever a new DNA size marker run has been performed, a new DNA reference marker table must be created.

For all other QIAxcel DNA Kits, a single DNA size marker reference table can be used for the entire life of the cartridge. However, the separation temperature and age of cartridge and buffer will introduce variations into DNA size determination. For optimal results, we recommend creating a new DNA reference marker table every 8 runs or after each 96-well plate. The DNA size marker reference table allows the size of sample DNA fragments to be determined without the need to run a QX DNA Size Marker every time.

Important points before starting

QIAxcel DNA Fast Analysis Kit

- Only 1 channel or capillary is required to generate the DNA size marker reference table. Ensure that the same method used to create the DNA reference marker table is used for sample analysis.
- Apply 10 μ l QX DNA Size Marker 50 bp – 1.5 kb (cat. no. 929554) to channel 1.

- Apply 10 μ l QX DNA Dilution Buffer (cat. no. 929601) to channels 2–12.

Note: A 10 μ l volume of QX DNA Dilution Buffer must be applied to each empty channel (i.e., channels into which no marker and no sample are loaded). Failure to do so may cause damage to empty capillary channels.

Note: QX DNA Size Marker 50 bp – 1.5 kb should be replaced every 50 runs or 3 days, whichever comes first. When not in use, store the 12-tube strip containing QX DNA Size Marker 50 bp – 1.5 kb at -20°C .

Important points before starting

QIAxcel DNA High Resolution Kit and QIAxcel DNA Screening Kit

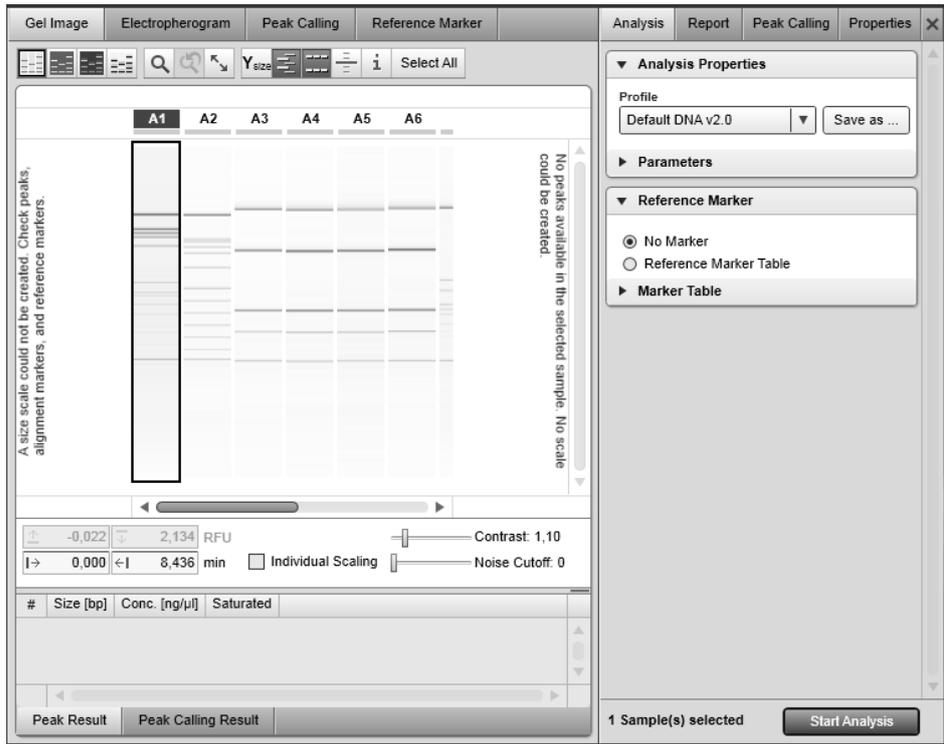
- Only 1 channel or capillary is required to generate the DNA size marker reference table.
- Only apply a reference marker table to a sample run using the same method and cartridge. The relative migration time (reltime) of the DNA size marker reference table is dependent on the separation voltage. Thus, the same separation voltage used to create the DNA size marker reference table must be used for the sample run. The normalized area of the peak is dependent on the injection voltage and the injection time. Thus, the same injection conditions used to create the DNA size marker reference table must be used for the sample run.
- For STR and microsatellite genotyping, a DNA size marker should be included in each sample run.
- The DNA size marker should be diluted in the same buffer as the sample to obtain optimal results (see “Sample preparation recommendations” page 18).

Procedure with QIAxcel ScreenGel Software

B1. Open the experiment to be analyzed.

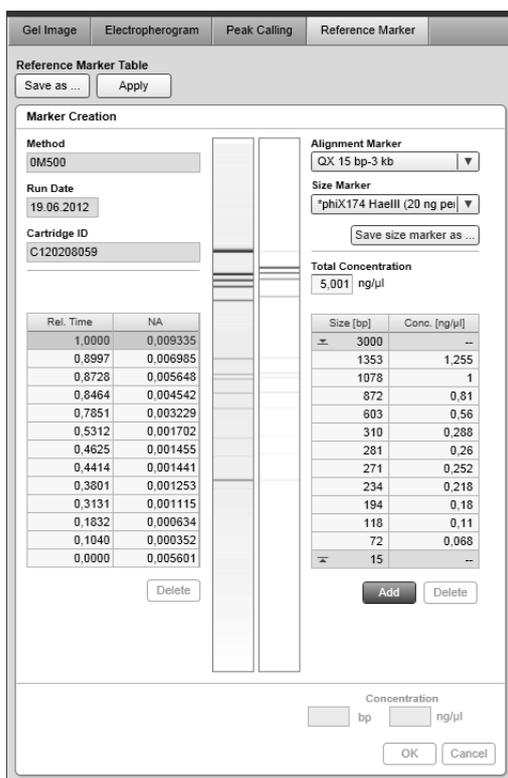
B2. Select the lane that was assigned as the size marker by clicking in the gel image that appears. If the size marker lane was not assigned, right-click the respective well in the experiment explorer to assign the size marker.

B3 Open the analysis tab, select “No Marker”, and click “Start Analysis”.



B4. Open the reference marker dialog box by opening the “Reference Marker” tab. From the drop-down list, select the size marker or create a new marker by selecting “New Size Marker”.

Note: The default total concentration of the preinstalled size markers, 20 ng/μl, can be modified to adjust to the actual dilution/concentration.



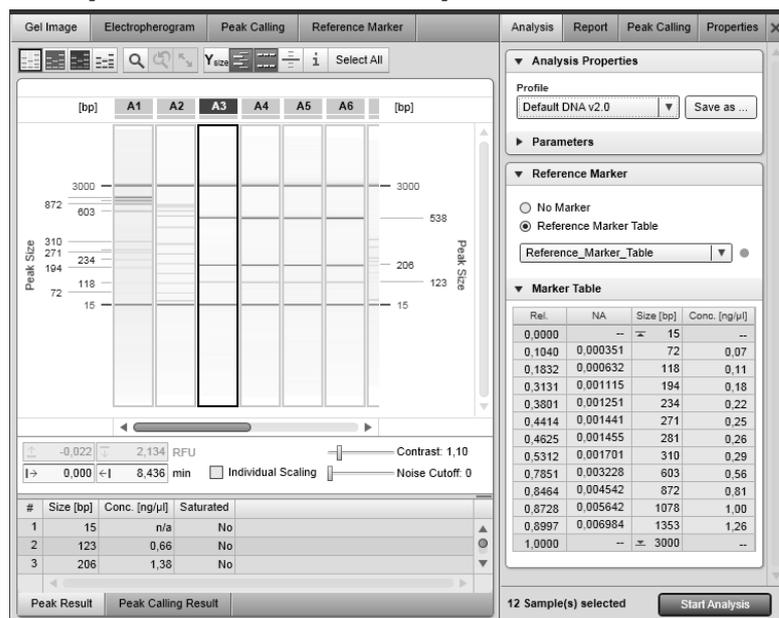
B5. Click “Apply” to create the DNA size marker reference table.

Note: If the number of peaks recognized by the software does not correspond to the size marker selection, check the electropherogram of the marker lane to be sure that all fragments were detected and make sure the right marker was selected from the drop-down list.

B6. Optional: Save as a reference marker for further use.

B7. Return to the gel image overview by opening the “Gel Image” tab.

B8. Click “Select all” or select lanes of interest and then click “Start Analysis” to start the analysis.



Procedure with BioCalculator software

B1. Open the data acquired for the DNA size marker channel by selecting the corresponding file from the file bar or by clicking the title bar of the corresponding dialog box, and maximize the window.

Ensure all peaks in the results table at the bottom of the dialog box are above the positive threshold setting (refer to Section 6.7.1 of the QIAxcel User Manual for more information).

B2. Select “Analysis” and then “Reference Markers” in the menu bar to display the “Reference Markers” dialog box.

B3. For the QIAxcel DNA Fast Analysis Kit, select “Size”, and for other QIAxcel DNA Kits, select “Size/Conc.” from the drop-down menu.

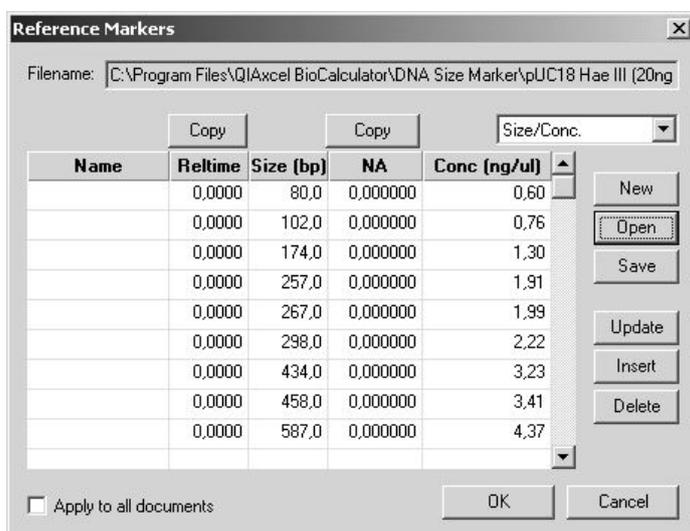
B4. Click “Open” to select a reference marker file.

Templates for reference marker files can be found on your computer in the **DNA Size Marker** folder (e.g., **C:\Program Files\QIAxcel BioCalculator\DNA Size Marker**).

Note: A custom DNA size marker reference table (i.e., allowing use of different DNA size markers) can be created by clicking “New” and entering the relevant information.

B5. Double-click the name of the reference DNA size marker file to be opened.

Alternatively, select the file to be opened, and click “Open”. The selected reference QX size marker data are shown in the “Reference Markers” dialog box.



Note: The “NA” and “Conc (ng/ul)” columns are not displayed for the QIAxcel DNA Fast Analysis Kit.

B6. When using the QX DNA Size Marker 50 bp – 1.5 kb with the QIAxcel DNA Fast Analysis Cartridge, continue with step B10.

B7. Position the cursor in the first row of the table, and click “Insert” to enter a new (blank) row above the specified rows.

B8. Enter the first peak value of the QX Alignment Marker in the “Size (bp)” field (i.e., 15 bp for the QX Alignment Marker 15 bp/5 kb, cat. no. 929524).

B9. Position the cursor in the last row of the table and enter the last peak value of the QX alignment marker (i.e., 5000 bp for the QX Alignment Marker 15 bp/5 kb, cat. no. 929524) in the “Size (bp)” field.

B10. For the QIAxcel DNA Fast Analysis Kit, click “Copy” (above “Reltime”) to copy the relative migration time from the result table to the reference marker table. For all other QIAxcel DNA Kits, click the “Copy” buttons above “Reltime” and “NA” to copy the relative migration time and normalized area from the result table to the reference marker table.

B11. Check “Apply to all documents”.

Note: Always ensure that the value for the first peak of the alignment marker in the “Reltime” column is 0 and the value for the last peak of the alignment marker is 1. The number of peaks in the data table acquired for channel 1 must be the same as the number of peaks in the reference marker table. Otherwise, the “Reltime” column will show an incorrect number of rows in the reference marker table, which will result in miscalculation of the DNA size and concentration.

B12. Click “Save”.

To use the reference marker for future analysis, we recommend saving the file under a different name that includes the name of the method used.

B13. Select “Analysis/Run” from the menu bar.

The fragment size and concentration will appear for each DNA fragment in each channel.

Determination of DNA length

Gel image format

The fragment sizes (bp) of the reference marker and/or the currently active lane are displayed on the sides of the gel image window depending on the ScreenGel software version. In addition, the estimated sizes of the sample DNA fragments are shown. DNA fragment sizes are displayed when the pointer is moved over a fragment.

Individual data

The size (bp) of the DNA fragment appears next to each peak in the electropherogram and in the “Size (bp)” column of the results table. The concentration of the DNA fragment appears only in the “Conc (ng/μl)” column of the results table.

Appendix C: QIAxcel Methods

QIAxcel High Resolution methods

The QIAxcel DNA High Resolution Kit is designed for rapid separation and analysis of DNA fragments ranging from 15 bp to 10 kb in size. The resolution depends on the fragment size and the method chosen to run the assay (see Table 3). The preinstalled methods that can be used with the QIAxcel DNA High Resolution Kit are listed in Tables 3–6.

Note: Amplification reactions contain dNTPs and primers, which can cause overestimation of the DNA concentration.

Table 3. Guidelines for method selection using the QIAxcel DNA High Resolution Kit

Method	Fragment size			
	100–500 bp	500 bp – 1 kb	1–5 kb	5–10 kb
	Best resolution			
OM400* OL400† OH400‡	20 bp	100 bp	500 bp	N/A
OM500* OL500† OH500‡	10 bp	50 bp	200 bp	N/A
OM800* OL800† OH800‡	3–5 bp	N/A	N/A	N/A
OM1200* OL1200† OH1200‡	N/A	N/A	500 bp – 1 kb	1–1.5 kb

* We recommend the OM400, OM500, OM700, and OM1200 methods for DNA concentrations of 10–100 ng/μl (e.g., PCR products amplified from genomic DNA with 30–40 cycles).

† We recommend the OL400, OL500, OL700, and OL1200 methods for DNA concentrations of < 10 ng/μl.

‡ We recommend the OH400, OH500, OH700, and OL1200 methods for DNA concentrations of > 100 ng/μl (e.g., high-yield PCR products).

M methods

We recommend the 0M400, 0M500, 0M700, 0M800, and 0L1200 methods for DNA concentrations of 10–100 ng/μl.

Table 4. M methods for use with the QIAxcel DNA High Resolution Kit

Method	Sample injection voltage (kV)	Sample injection time (s)*	Separation voltage (kV)	Separation time (s)
0M400	5	10	6	400
0M500	5	10	5	500
0M700	5	10	3	700
0M800	5	10	3	800
0M1200	5	10	3.5	1200

* Sample injection time can be adjusted (minimum 1 s; maximum 60 s). Reduce the injection time if the signal is saturated, as indicated by peaks with flat tops. Increase sample injection time if the signal is below the default setting of the positive threshold of 5%.

L methods

We recommend the 0L400, 0L500, 0L700, 0L800, and 0M1200 methods for DNA concentrations of <10 ng/μl.

Table 5. L methods for use with the QIAxcel DNA High Resolution Kit

Method	Sample injection voltage (kV)	Sample injection time (s)*	Separation voltage (kV)	Separation time (s)
0L400	8	20	6	400
0L500	8	20	5	500
0L700	8	20	3	700
0L800	8	20	3	800
0L1200	8	20	3.5	1200

* Sample injection time can be adjusted (minimum 1 s; maximum 60 s). Reduce the injection time if the signal is saturated, as indicated by peaks with flat tops. Increase sample injection time if the signal is below the default setting of the positive threshold of 5%.

H Methods

We recommend the 0H400, 0H500, 0H700, 0H800, and 0H1200 methods for DNA concentrations of >100ng/ μ l.

Table 6. H methods for use with the QIAxcel DNA High Resolution Kit

Method	Sample injection voltage (kV)	Sample injection time (s)*	Separation voltage (kV)	Separation time (s)
0H400	2	20	6	400
0H500	2	20	5	500
0H700	2	20	3	700
0H800	2	20	3	800
0H12000	2	20	3.5	1200

* Sample injection time can be adjusted (minimum 1 s; maximum 60 s). Reduce the injection time if the signal is saturated, as indicated by peaks with flat tops. Increase sample injection time if the signal is below the default setting of the positive threshold of 5%.

QIAxcel DNA Screening methods

The QIAxcel DNA Screening Kit is for low-resolution (>20bp) genotyping, low-resolution multiplex PCR, PCR screening, analysis of plasmid DNA restriction digests, and determination of amount of plasmid or oligo DNA. The gel cartridge can separate fragments from 15 bp to 5 kb. The resolution depends on the fragment size and the method chosen to run the assay (see Table 7). The preinstalled methods that can be used with the QIAxcel DNA Screening Kit are listed in Tables 7–10).

Note: Amplification reactions contain dNTPs and primers, which can cause overestimation of the DNA concentration.

Table 7. Guidelines for method selection using the QIAxcel DNA Screening Kit

Method	Fragment size		
	<500 bp	500 bp – 1 kb	1–5 kb
	Best resolution		
AM320*	20 bp	100 bp	500 bp
AL320†			
AH320‡			
AM420*	20 bp	100 bp	500 bp
AL420†			
AH420‡			
APH600	Analysis of uncut plasmid DNA		
APL600			

* We recommend the AM320 and AM420 methods for DNA concentrations of 10–100 ng/μl (e.g., PCR products amplified from genomic DNA with 30–40 cycles).

† We recommend the AL320 and AL420 methods for DNA concentrations of < 10 ng/μl.

‡ We recommend the AH320 and AH420 methods for DNA concentrations of > 100 ng/μl (e.g., high-yield PCR products).

M methods

We recommend the AM320 and AM420 methods for DNA concentrations of 10–100 ng/μl and the method APH600 for purified high-copy plasmid DNA (50–300 ng/μl) in elution buffer.

Table 8. M methods for use with the QIAxcel DNA Screening Kit

Method	Sample injection voltage (kV)	Sample injection time (s)*	Separation voltage (kV)	Separation time (s)
AM320	5	10	6	320
AM420	5	10	5	420

* Sample injection time can be adjusted (minimum 1 s; maximum 60 s). Reduce the injection time if the signal is saturated, as indicated by peaks with flat tops. Increase sample injection time if the signal is below the default setting of the positive threshold of 5%.

L Methods

We recommend the AL320 and AL420 methods for DNA concentrations of <10 ng/ μ l and the method APL600 for purified low-copy plasmid DNA (<50 ng/ μ l) in elution buffer.

Table 9. L methods for use with the QIAxcel DNA Screening Kit

Method	Sample injection voltage (kV)	Sample injection time (s)*	Separation voltage (kV)	Separation time (s)
AL320	8	20	6	320
AL420	8	20	5	420
APL600	3	5	6	600

* Sample injection time can be adjusted (minimum 1 s; maximum 60 s). Reduce the injection time if the signal is saturated, as indicated by peaks with flat tops. Increase sample injection time if the signal is below the default setting of the positive threshold of 5%.

H Methods

We recommend the AH320 and AH420 methods for DNA concentrations of >100ng/ μ l.

Table 10. H methods for use with the QIAxcel DNA Screening Kit

Method	Sample injection voltage (kV)	Sample injection time (s)*	Separation voltage (kV)	Separation time (s)
AH320	2	20	6	320
AH420	2	20	5	420
APH600	1	5	6	600

* Sample injection time can be adjusted (minimum 1 s; maximum 60 s). Reduce the injection time if the signal is saturated, as indicated by peaks with flat tops. Increase sample injection time if the signal is below the default setting of the positive threshold of 5%.

QIAxcel DNA Fast Analysis methods

The QIAxcel DNA Fast Analysis Kit is designed for rapid analysis of PCR fragments. The gel cartridge can separate fragments ranging from 15 bp to 3 kb in size (see Table 11). The preinstalled methods that can be used with the QIAxcel DNA Fast Analysis Kit are shown in Table 11.

Note: The QIAxcel DNA Fast Analysis Cartridge, QX DNA Size Marker 50 bp – 1.5 kb, and the corresponding methods are not suited for concentration determination. If concentration determination is required, we recommend DM80 v2.0, DM80, and DM150.

Generally, runs on the QIAxcel DNA Fast Analysis Cartridge have lower resolution compared to alternative QIAxcel DNA Cartridges. For the most rapid PCR fragment analysis, with the lowest resolution, we recommend DM80 v2.0. If the resolution using DM80 v2.0 is not sufficient, we recommend DM150.

Table 11. Methods for use with the QIAxcel DNA Fast Analysis Kit

Method	Sample injection voltage (kV)	Sample injection time (s)*	Separation voltage (kV)	Separation time (s)
DM80	15	8	15	80
DM 80 v2.0 [†]	15	8	15	80
DM150	10	10	10	150

* Sample injection time can be adjusted (minimum 1 s; maximum 40 s). To obtain optimal signals, we recommend using a minimum of 5 s and maximum of 15 s.

[†] DM80 v2.0 includes a longer purge time between runs and thus improves the background level.

Appendix D: Removing Gel from Blocked Capillaries

Blocked capillaries may lead to issues during calibration or sample runs. Gel can be removed from blocked capillaries by performing the standard gel-droplet test. If the standard gel-droplet test fails, gel can be removed by performing the gel-droplet test with hot water or by performing the gel-droplet test using the QX Cartridge Prep Station. After confirmation of gel flow, a signal check can be performed before starting a calibration or sample run (see page 49).

Standard gel-droplet test

Note: The QIAxcel gel cartridge must be prepared before the standard gel-droplet test is performed. Prepare the QIAxcel gel cartridge by following steps 1–4 on page 13.

Procedure with QIAxcel ScreenGel software

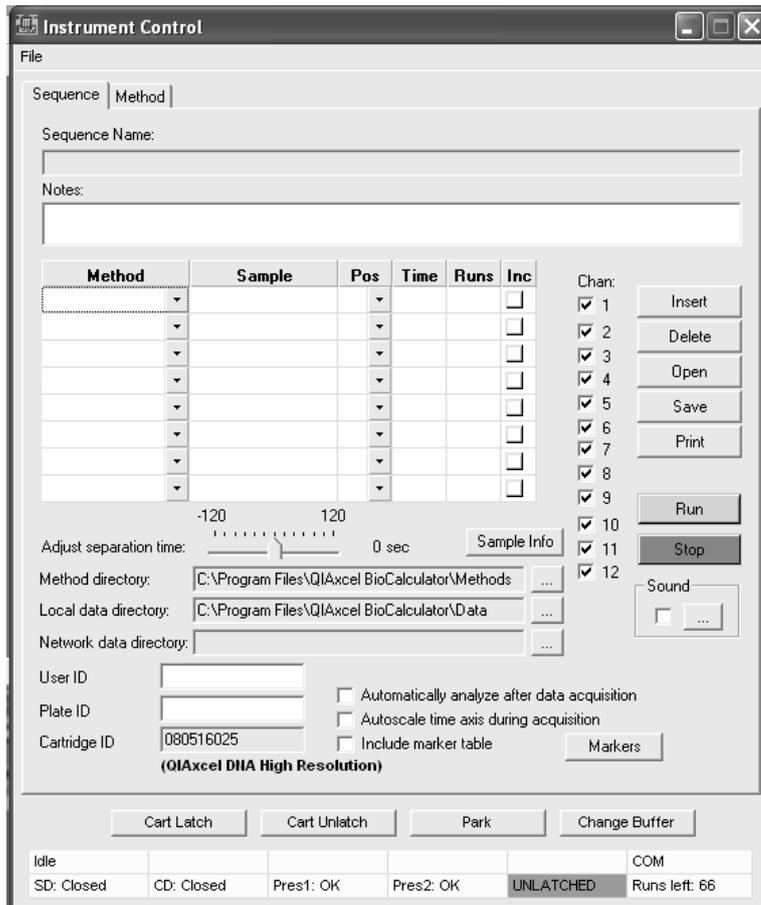
- D1. Launch the QIAxcel ScreenGel software, and click “Load Position” in the status information bar.**
- D2. Remove the buffer tray from the buffer tray holder.**
- D3. Place a soft tissue on the buffer tray holder and click “Park Position”.**
- D4. Install the QIAxcel gel cartridge and the smart key as described in Section 5.2.3 of the *QIAxcel User Manual* or the *QIAxcel Advanced User Manual*.**
- D5. Ensure that the cartridge identifier is displayed in the “Cartridge Status” window.**
- D6. Click “Latch” to latch the cartridge if automatic latching is not active.**
- D7. Switch to the “Service” Environment, and open the maintenance tab.**
- D8. Click “Long Purge” to start purging.**

The purging process stops automatically after 3 min. To stop the purging process, click “Stop”.
- D9. Open the sample door. If all capillaries have formed homogeneous gel droplets, click “Unlatch”. Remove the gel cartridge from the instrument and carefully clean the capillary tips with a wet tissue.**

Note: If any capillary fails to form gel droplets, perform the gel-droplet test with hot water.
- D10. Remove the tissue from the buffer tray holder and insert the buffer tray into the buffer tray holder.**
- D11. Place the gel cartridge into the QIAxcel instrument and perform the signal check (see page 49).**

Procedure with BioCalculator software

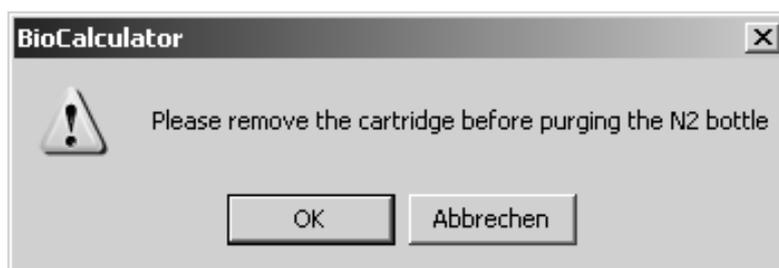
- D1. Launch the BioCalculator software, and click “Change Buffer” in the “Instrument Control” window.
- D2. Remove the buffer tray from the buffer tray holder.
- D3. Place a soft tissue on the buffer tray holder, and click “Park Position”.
- D4. Install the QIAxcel gel cartridge and the smart key as described in Section 5.2.3 of the *QIAxcel User Manual*.
- D5. Ensure that the cartridge identifier is displayed in the “Instrument Control” window.



D6. Click “Cart Latch”.

D7. Open File/Settings and then click “Purge”.

The window shown below opens.



D8. Leave the QIAxcel gel cartridge in the instrument (ignore the warning message), and click “OK” to start the purging process.

The purging process stops automatically after 3 min. To stop the purging process, click “Purge” again.

D9. Open the sample door. If all capillaries have formed homogeneous gel droplets, click “Cart Unlatch”. Remove the gel cartridge from the instrument and carefully clean the capillary tips with a wet tissue.

Note: If any capillary fails to form gel droplets, perform the gel-droplet test with hot water.

D10. Remove the tissue from the buffer tray holder and insert the buffer tray into the buffer tray holder.

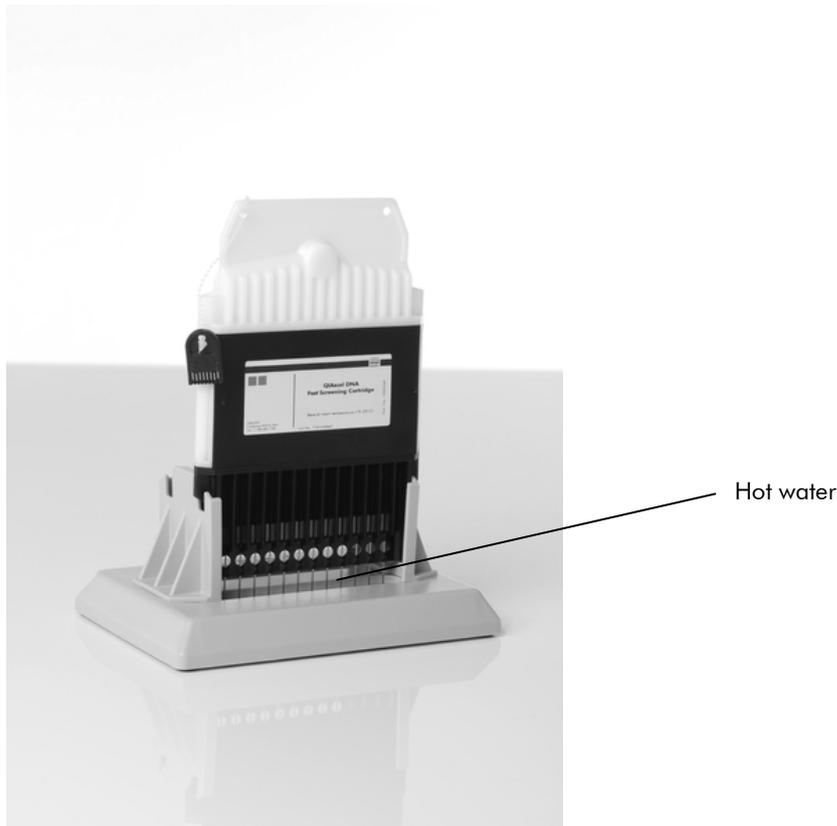
D11. Place the gel cartridge inside the QIAxcel instrument and perform the manual signal check (see page 49).

Gel-droplet test with hot water

If any of the capillaries fail to form droplets in the standard gel-droplet test, clogged capillaries can be cleared by performing a purge test with hot water.

Procedure

D1. If any of the capillaries fail to form droplets in the standard gel-droplet test (page 44), fill the reservoir of the QX Cartridge Stand (cat. no. 929708) with 12 ml hot (90°C or near boiling) water.



- D2. Place the cartridge in the reservoir of the QX Cartridge Stand and submerge the tips for 7–10 min in the hot water. This should soften the dried gel at the capillary tips.**
- D3. Empty the reservoir and fill again with 12 ml hot water.**
- D4. Repeat step D2 to soften any dried gel at the capillary tips.**
- D5. Place the cartridge into the QIAxcel instrument and perform the standard gel-droplet test again (see page 44).**
- D6. Check whether gel droplets form at the capillary tips. If any of the capillaries still do not form homogeneous gel droplets, submerge the capillary tips in hot water for 20–30 min and then repeat step D5.**
- D7. If all capillaries form homogeneous gel droplets, clean the tips with wet tissue, and perform the manual signal check (see page 49). If after 3 attempts a capillary fails to form homogeneous gel droplets, contact QIAGEN Technical Services or perform the gel droplet test using the QX Cartridge Prep Station.**

Gel-droplet test using the QX Cartridge Prep Station

If after 3 attempts homogeneous droplets do not form for all capillaries in the gel-droplet test with hot water (page 46), clogged capillaries can be cleared by performing a purge test in the QX Cartridge Prep Station (cat. no. 9018886).

- D1. Place the QIAxcel gel cartridge in the QX Cartridge Prep Station (cat. no. 9018886).**
- D2. Attach the purge port clamp to the top of the cartridge (the knob should be in front of the cartridge), and gently tighten the knob to secure the cartridge.**
- D3. Insert a QX Nitrogen Cylinder (cat. no. 929705) into the pressure regulator (cat. no. 9018398), and secure it inside the cylinder stand.**



D4. Slowly adjust the pressure to 60–65 psi, monitoring the pressure displayed in the pressure gauge.

Do not exceed 65 psi.

D5. Check to see whether gel droplets form at the capillary tips.

D6. If all capillaries formed homogeneous gel droplets within 1–3 min, turn off the pressure, remove the purge clamp and clean the tips with wet tissue.

D7. Place the cartridge into the instrument, and perform the manual signal check (see below).

D8. If any of the capillaries fail to form gel droplets within 1–3 min, contact QIAGEN Technical Services.

Performing a signal check

The performance of the cartridge channels can be tested by performing a signal check. The signal check should be performed successfully (i.e., all channels should detect a single peak at 1.0–3.5 minutes) before running the calibration wizard again.

Things to do before starting

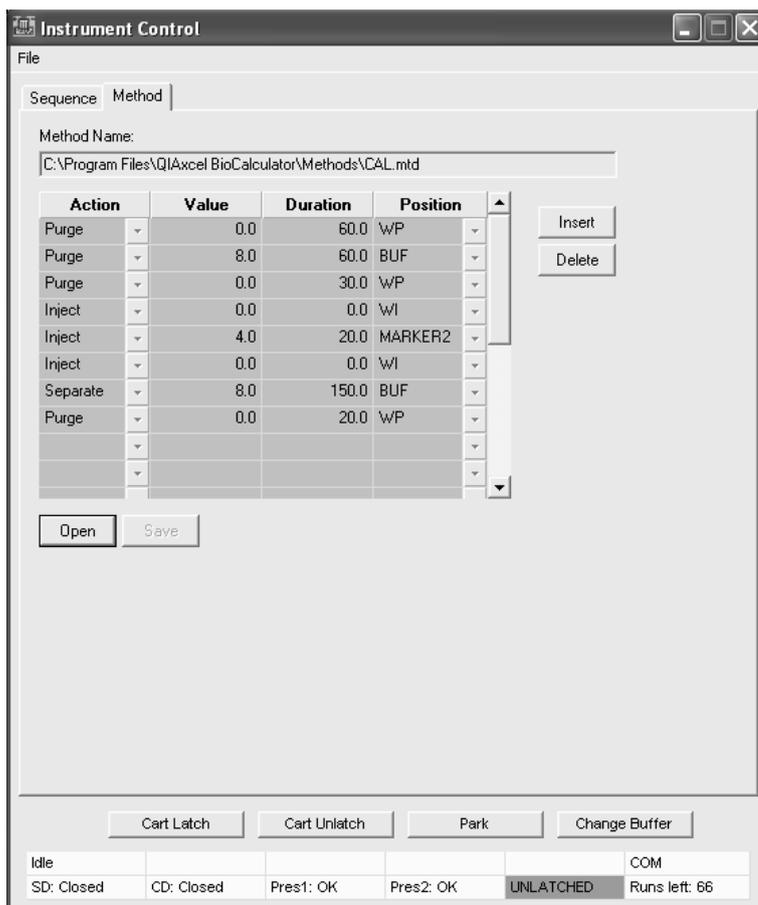
- Prepare the buffer tray as described in Section 5.2.1 of the *QIAxcel User Manual* or the *QIAxcel Advanced User Manual*.
- Load the buffer tray as described Section 5.2.2 of the *QIAxcel User Manual* or the *QIAxcel Advanced User Manual*.
- Load 15 μ l of the QX Intensity Calibration Marker into the MARKER2 position of the buffer tray (see “Preparing the QIAxcel gel cartridge and buffer tray”, page 12 for more information).

Procedure using QIAxcel ScreenGel Software

- D1. Launch the QIAxcel ScreenGel Software.**
- D2. From the drop-down list in the process profile tab, select the “Signal Check” process profile that corresponds to the cartridge in use.**
- D3. Click the “Run Check” tab, enter the required information, and click “Run”.**
- D4. If a single peak is detected in all channels, intensity calibration can be performed again (see “Intensity calibration”, page 16). If one or more channels fail to detect a peak (i.e., no band present), contact QIAGEN Technical Services.**

Procedure using BioCalculator software

- D1. Launch the BioCalculator software.**
- D2. Select the Cal.mtd method from the method drop-down list in the “Instrument Control” window.**
- D3. To view the method parameters, select the “Method” tab.**



D4. To run the method, select the “Sequence” tab and click “Run”.

D5. If a single peak is detected in all channels, perform intensity calibration (see “Intensity calibration”, page 16). If one or more channels fail to detect a peak (i.e., no band present), contact QIAGEN Technical Services.

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.

Ordering Information

Product	Contents	Cat. no.
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QIAxcel DNA Screening Kit (2400)	QIAxcel DNA Screening Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QIAxcel DNA Fast Analysis Kit (3000)	QIAxcel DNA Fast Analysis Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX DNA Size Marker 50 bp – 1.5 kb, QX Alignment Marker 15 bp/3 kb, 12-Tube Strips	929008
QIAxcel RNA QC Kit v2.0 (1200)	For 100 runs of 12 samples: QIAxcel RNA Quality Control Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX RNA Alignment Marker, QX RNA Size Marker 200–6000 nt, QX RNA Denaturation Buffer, 12-Tube Strips	929104
QIAxcel Advanced System	Capillary electrophoresis device, including computer, and QIAxcel ScreenGel Software; 1-year warranty on parts and labor	9001941
Warranty PLUS 2 Basic, QIAxcel	3-year warranty, response time of 5-working days, all labor, travel, and repair parts	9241202
Warranty PLUS 2 Full, QIAxcel	3-year warranty, 48-hour (2 working days) priority response, all labor, travel, and repair parts	9241330
Software		
QIAxcel ScreenGel Software	Separate license for use of QIAxcel ScreenGel Software on an additional computer	9021163
QIAxcel ScreenGel Software (10)	10 separate licenses for use of QIAxcel ScreenGel Software on additional computers	9021165

Product	Contents	Cat. no.
QX Size Markers		
QX DNA Size Marker pUC18/HaeIII (50 μ l)	DNA size marker with fragments of 80, 102, 174, 257, 267, 298, 434, 458, and 587 bp; concentration 100 ng/ μ l	929550
QX DNA Size Marker 25 bp – 1.8 kb (50 μ l)	DNA size marker with fragments of 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, and 1800 bp; concentration 100 ng/ μ l	929552
QX Size Marker 25 bp – 500 bp (50 μ l) v2.0	DNA size marker with fragments of 25, 50, 75, 100, 150, 200, 250, 300, 400, and 500 bp; concentration 100 ng/ μ l	929560
QX DNA Size Marker 100 bp – 2.5 kb (50 μ l)	DNA size marker with fragments of 100, 200, 300, 400, 500, 600, 700, 800, 1000, 1200, 1500, 2000, and 2500 bp; concentration 100 ng/ μ l	929559
QX DNA Size Marker 50 bp – 1.5 kb (100 μ l)	DNA size marker with fragments of 50, 100, 200, 300, 400, 500, 1000, and 1500 bp; ready-to-use	929554
QX DNA Size Marker 50–800 bp (50 μ l) v.2.0	DNA size marker with fragments of 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, and 800 bp; concentration 100 ng/ μ l	929561
QX DNA Size Marker 250 bp – 4 kb (50 μ l) v.2.0	DNA size marker with fragments of 250, 500, 750, 1000, 1200, 1500, 2000, 2500, 3000, 3500, and 4000 bp; concentration 100 ng/ μ l	929562
QX DNA Size Marker 250 bp – 8 kb (50 μ l) v.2.0	DNA size marker with fragments of 250, 500, 750, 1000, 2000, 3000, 4000, 5000, 6000, and 8000 bp; concentration 100 ng/ μ l	929563
QX Alignment Markers		
QX Alignment Marker 15 bp/500 bp (1.5 ml)	Alignment marker with 15 bp and 500 bp fragments	929520

Product	Contents	Cat. no.
QX Alignment Marker 15 bp/600 bp (1.5 ml)	Alignment marker with 15 bp and 600 bp fragments	929530
QX Alignment Marker 15 bp/1 kb (1.5 ml)	Alignment marker with 15 bp and 1 kb fragments	929521
QX Alignment Marker 15 bp/3 kb (1.5 ml)	Alignment marker with 15 bp and 3 kb fragments	929522
QX Alignment Marker 15 bp/5 kb (1.5 ml)	Alignment marker with 15 bp and 5 kb fragments	929524
QX Alignment Marker 15 bp/10 kb (1.5 ml)	Alignment marker with 15 bp and 10 kb fragments	929523
QX Alignment Marker 50 bp/1 kb (1.5 ml)	Alignment marker with 50 bp and 1 kb fragments	929526
QX Alignment Marker 50 bp/5 kb (1.5 ml)	Alignment marker with 50 bp and 5 kb fragments	929529
QX Calibration Marker		
QX Intensity Calibration Marker (1 ml)	1 ml QX Intensity Calibration Marker	929500
QX Buffers		
QX DNA Dilution Buffer (15 ml)	15 ml QX DNA Dilution Buffer	929601
QX Separation Buffer (40 ml)	40 ml QX Separation Buffer	929603
QX FA Separation Buffer (40 ml)	40 ml QX FA Separation Buffer	929606
QX Wash Buffer (40 ml)	40 ml QX Wash Buffer	929604
QX Mineral Oil (50 ml)	50 ml QX Mineral Oil	929605
QIAxcel Accessories		
QX Cartridge Stand with Cover	QX Cartridge Stand and QX Cartridge Stand Cover	929708

Product	Contents	Cat. no.
QX Buffer Tray	Buffer tray for use with the QIAxcel system	929702
QX 0.2 ml 12-Tube Strip (80)	80 x QX 0.2 ml 12-Tube Strips	929703
QX Color 0.2 ml 12-Tube Strip (80)	80 x QX Color 0.2 ml 12-Tube Strips	929704
QX 0.2 ml 12-Tube Strip Caps (80)	80 strip caps for use with QX 0.2 ml 12-Tube Strips	929706
QX Nitrogen Cylinder (6)	6 QIAxcel Nitrogen Cylinders	929705

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