

# DyeEx™ Handbook

For

**NEW** DyeEx 2.0 Spin Kit  
DyeEx 96 Kit

May 2002



## QIAGEN Worldwide

---

### QIAGEN Companies

<b>Australia</b>	<b>QIAGEN Pty Ltd</b> ABN 75 072 382 944	PO Box 25 • Clifton Hill • Victoria 3068 Orders 03-9489-3666 • Fax 03-9489-3888 • Technical 1-800-243-066
<b>Canada</b>	<b>QIAGEN Inc.</b>	2800 Argentia Road • Unit 7 • Mississauga • Ontario • L5N 8L2 Orders 800-572-9613 • Fax 800-713-5951 • Technical 800-DNA-PREP (800-362-7737)
<b>France</b>	<b>QIAGEN S.A.</b>	3 avenue du Canada • LP 809 • 91974 COURTABOEUF CEDEX Orders 01-60-920-920 • Fax 01-60-920-925 • Technical 01-60-920-930
<b>Germany</b>	<b>QIAGEN GmbH</b>	Max-Volmer-Straße 4 • 40724 Hilden Orders 02103-29-12000 • Fax 02103-29-22000 • Technical 02103-29-12400
<b>Italy</b>	<b>QIAGEN S.p.A.</b>	Via Grosio, 10/10 • 20151 Milano Orders 02-33430411 • Fax 02-33430426 • Technical 02-33430414
<b>Japan</b> <a href="http://www.qiagen.co.jp">www.qiagen.co.jp</a>	<b>QIAGEN K.K.</b>	Forefront Tower II • 13-1, Kachidoki 3 Chome • Chuo-ku, Tokyo 104-0054 Telephone 03-5547-0811 • Fax 03-5547-0818 • Technical 03-5547-0811
<b>Switzerland</b>	<b>QIAGEN AG</b>	Auf dem Wolf 39 • 4052 Basel Orders 061-319-30-30 • Fax 061-319-30-33 • Technical 061-319-30-31
<b>UK and Ireland</b>	<b>QIAGEN Ltd.</b>	Boundary Court • Gatwick Road • Crawley • West Sussex, RH10 9AX Orders 01293-422-911 • Fax 01293-422-922 • Technical 01293-422-999
<b>USA</b>	<b>QIAGEN Inc.</b>	28159 Avenue Stanford • Valencia • CA 91355 Orders 800-426-8157 • Fax 800-718-2056 • Technical 800-DNA-PREP (800-362-7737)

[www.qiagen.com](http://www.qiagen.com)

### QIAGEN Distributors

Please see the last page for contact information for your local QIAGEN distributor.

# Contents

<b>Kit Contents</b>	<b>4</b>
<b>Storage Conditions</b>	<b>4</b>
<b>Quality Control</b>	<b>4</b>
<b>Technical Assistance</b>	<b>5</b>
<b>Product Use Limitations</b>	<b>5</b>
<b>Product Warranty and Satisfaction Guarantee</b>	<b>5</b>
<b>Introduction</b>	<b>6</b>
<b>Protocol for Dye-Terminator Removal Using:</b>	
■ <b>DyeEx 2.0 Spin Kit</b>	<b>8</b>
■ <b>DyeEx 96 Kit</b>	<b>10</b>
Standard Protocol for ABI PRISM 310, 373, 3100, 377, MegaBACE 1000	12
Modified Protocol for ABI PRISM 3700 and Beckman CEQ 2000	14
<b>Troubleshooting Guide</b>	<b>16</b>
<b>Appendix</b>	<b>18</b>
DyeEx principle	18
Special application for labeling-reaction cleanup	18
<b>Ordering Information</b>	<b>21</b>
<b>QIAGEN Distributors</b>	<b>23</b>

## Kit Contents

<b>DyeEx™ 2.0 Spin Kit</b>	<b>(50)</b>	<b>(250)</b>
<b>Catalog No.</b>	<b>63204</b>	<b>63206</b>
<hr/>		
DyeEx™ 2.0 Spin Columns	50	250
Collection Tubes (2 ml)	50	250
Handbook	1	1
<b>DyeEx 96 Kit</b>	<b>(4)</b>	<b>(24)</b>
<b>Catalog No.</b>	<b>63181</b>	<b>63183</b>
<hr/>		
DyeEx 96 Plates	4	24
Waste Collection Plates, 48-well	4	4
Handbook	1	1

## Storage Conditions

DyeEx 2.0 Spin Kits should be stored dry at room temperature (15–25°C). Under these conditions, these kits can be stored for up to 12 months without showing any reduction in performance and quality. For longer storage, these kits can be stored at 2–8°C. Do not freeze.

DyeEx 96 Kits should be stored at 2–8°C. Do not freeze. Under these conditions, these kits can be stored for up to 12 months without showing any reduction in performance and quality.

## Quality Control

As part of the stringent QIAGEN® quality assurance program, the performance of DyeEx 2.0 Spin Kits is monitored routinely on a lot-to-lot basis. Removal of dye terminators is tested by determining the presence of contaminating signals in sequencing profiles obtained following cleanup of cycle sequencing reactions. Sequence signal intensities and read lengths are also evaluated. In addition, both the particle size and quantity of gel-filtration material per column are tested.

The performance of DyeEx 96 Kits is also routinely monitored. Removal of dye terminators is tested by determining the presence of contaminating signals in sequencing profiles obtained following cleanup of cycle sequencing reactions. Sequence signal intensities and read lengths are also evaluated. In addition, particle size, and quantity of gel-filtration material per well are tested, and sealing of the plates is checked.

## Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding DyeEx Kits or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors listed on the last page.

## Product Use Limitations

DyeEx Kits are developed, designed, and sold for research purposes only. They are not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised when handling many of the materials described in this text.

## Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department. We will credit your account or exchange the product — as you wish.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor.

# Introduction

DyeEx Kits are designed for fast and easy removal of unincorporated dye terminators directly from sequencing reactions. DyeEx modules contain prehydrated gel-filtration resin and are ready to use. The kits have been optimized for cleanup of sequencing reactions containing any dye terminators, such as dRhodamine, DYEnamic ET, and particularly BigDye™ terminators, including v 3.0. A choice of spin column or 96-well plate format kits is available. Sequencing reaction products purified using DyeEx Kits can be analyzed with various DNA sequencers (Table 1).

## DyeEx 2.0 Spin Kit

The DyeEx 2.0 Spin Kit uses gel-filtration technology in a convenient microspin format allowing cleanup of sequencing reactions in just 7 minutes.

## DyeEx 96 Kit

The DyeEx 96 Kit allows efficient removal of dye terminators in a high-throughput 96-well format. Using four plates in a suitable centrifuge, 384 samples can be processed in 18 minutes using the standard protocol (see page 12). We recommend the first DyeEx 96 protocol (page 12) for sequence analysis using ABI PRISM 310, 373, or 377, or MegaBACE™ 1000 sequencers, while an optimized protocol has been developed to provide maximum signal intensities with the ABI PRISM 3700 DNA Analyzer or Beckman CEQ™ 2000 which have electrokinetic injection loading systems (page 14).

**Table 1. DyeEx Kit specifications**

<b>Specification</b>	<b>DyeEx Kit</b>
<b>Maximum sample volume</b>	<b>20 µl</b>
<b>Terminators removed</b>	
BigDye (including BigDye Terminators v. 3.0)	YES
dRhodamine dye	YES
Rhodamine dye	YES
DYEnamic ET	YES
WellRED dye	YES
<b>DNA sequencers</b>	<b>ABI PRISM® 377, 373, 310, 3100, 3700*, MegaBACE 1000, CEQ 2000*</b>

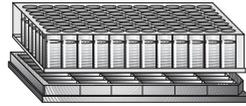
\* We recommend the DyeEx 96 Kit using the optimized protocol for the ABI PRISM 3700 sequencer and CEQ 2000.

## DyeEx 2.0 Spin Kit Procedure

## DyeEx 96 Kit Procedure



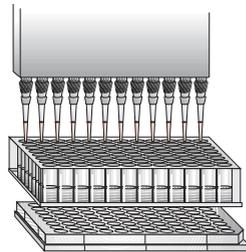
DyeEx 2.0 spin column  
in collection tube



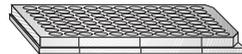
DyeEx plate on waste  
collection plate



Add sequencing  
reaction



Purified sequencing reactions



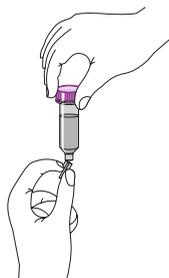
## DyeEx 2.0 Spin Protocol for Dye-Terminator Removal

### Important notes before starting

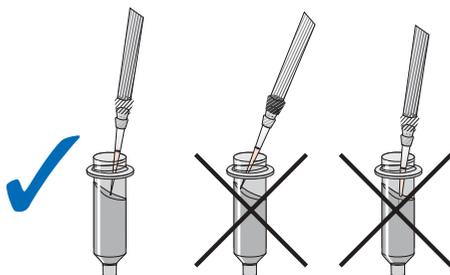
- We recommend the following protocol for sequence analysis using ABI PRISM 310, 3100, 370, 377, 3700, or Beckman CEQ 2000 sequencers.
- All centrifugation steps are performed at  $750 \times g$  in a conventional microcentrifuge. The appropriate speed for individual centrifuges can be calculated as follows:  $\text{rpm} = 1000 \times \sqrt{750/1.12 \cdot r}$  ( $r$  = radius of rotor in mm).

**Table 2. Examples of suitable microcentrifuges and the corresponding speeds**

Microcentrifuge	Speed
Eppendorf Centrifuge 5415C	3000 rpm
Eppendorf Centrifuge 5417C	2700 rpm
Heraeus Biofuge 15	2800 rpm
Hettich Mikro 24-48	2630 rpm
Beckman GS15R	2100 rpm
Hettich Mikro EBA12	2700 rpm



**Figure 1.** Snapping off the bottom closure of the DyeEx 2.0 spin column (do not screw).



**Figure 2.** Instructions for sample application to the DyeEx 2.0 spin column.

1. **Gently vortex the spin column to resuspend the resin.**
2. **Loosen the cap of the column a quarter turn.**

This is necessary to avoid a vacuum inside the spin column.
3. **Snap off the bottom closure of the spin column (Figure 1), and place the spin column in a 2 ml collection tube (provided).**
4. **Centrifuge for 3 min at the calculated speed.**
5. **Carefully transfer the spin column to a clean centrifuge tube. Slowly apply the sequencing reaction (10–20  $\mu$ l) to the gel bed (Figure 2).**

**Notes:**

- Pipet the sequencing reaction directly onto the center of the slanted gel-bed surface (Figure 2). Do not allow the reaction mixture or the pipet tip to touch the sides of the column. The sample should be pipetted slowly so that the drops are absorbed into the gel and do not flow down the sides of the gel bed. Avoid touching the gel-bed surface with the pipet tip.

- This protocol is suitable for sequencing reactions with volumes of 10–20  $\mu$ l. For easier handling, more reproducible pipetting, and reduced error with sample volumes <10  $\mu$ l, we recommend adjusting the volume to 20  $\mu$ l using distilled water, before application to the gel-bed.
- It is not necessary to remove mineral oil or kerosene prior to cleanup of dye-terminator sequencing reactions.
- It is not necessary to replace the lid on the column.

6. **Centrifuge for 3 min at the calculated speed.**
7. **Remove the spin column from the microcentrifuge tube.**

The eluate contains the purified DNA.

**Optional:** If using the ABI PRISM 3700 with a water loading protocol, it is possible to load the eluate directly onto the sequencer without drying down the sample.

8. **Dry the sample in a vacuum centrifuge and proceed according to the instructions provided with the DNA sequencer.**

# DyeEx 96 Protocol for Dye-Terminator Removal

## Important notes before starting

- Choose the appropriate protocol for your DNA sequencer:

### Standard protocol (see page 12)

This protocol is optimized for use with the ABI PRISM 310, 3100, 373, 377, or MegaBACE 1000 sequencing machines, assuring maximal signal intensity and read length. (It can also be used with the ABI PRISM 3700 and CEQ 2000, but a decrease in signal intensity may be observed, although the read length will typically stay the same.)

### Modified protocol (see page 14)

This protocol is optimized to provide the maximum signal intensity when using the ABI PRISM 3700 or Beckman CEQ 2000.

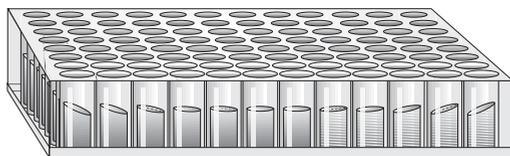
**Table 3. Examples of suitable centrifuges, rotors, adapters, and the corresponding speeds**

Centrifuge	Rotor	Adapter	Speed
QIAGEN Centrifuge 4-15C or 4K-15C*	Plate Rotor 2 x 96	Included	2500 rpm
Beckman Allegra 6 <sup>†</sup>	PTS-2000	Included	2300 rpm
Eppendorf 5810 <sup>†</sup>	MTP Rotor A-4-62	4 MTP adapters	2400 rpm
Heraeus Megafuge 1.0 <sup>†</sup>	Cat. No. 75002704	Microplate carrier (Cat. No. 75007586)	2500 rpm
Heraeus Megafuge 2.0 <sup>†</sup>	Cat. No. 75008155	Microplate carrier (Cat. No. 75008083)	2400 rpm
Hettich ROTANTA 96 or 46 <sup>†</sup>	Cat. No. 4444	4 MTP adapters	2300 rpm
Hettich ROTIXA 120 or 150S <sup>†</sup>	Cat. No. 4294	4 MTP adapters	2300 rpm
Sorvall T-6000 <sup>†</sup>	H-1000B	Microplate Carrier	2450 rpm
Sorvall RT7 <sup>†</sup>	RTH-250	Microplate Carrier	2450 rpm
Centra CL3/CLR <sup>†</sup>	Rotor 244	Included	4000 rpm
Multi/Multi RF <sup>†</sup>	Rotor 8244	Included	4000 rpm

\* Available from QIAGEN, for details contact QIAGEN Technical Services or your local distributor. This centrifuge is fully compatible with and highly recommended for use with the DyeEx 96 Kit.

<sup>†</sup> The centrifugation speeds have been calculated from information provided by the suppliers of the centrifuges. These centrifuges, rotors, and adapters have not been tested by QIAGEN. QIAGEN accepts no responsibility for the accuracy of the data given.

- A multichannel pipet facilitates handling of sequencing samples.
- The use of DyeEx 96 plates requires a suitable centrifuge, rotor, and adapters. Rotor and adapters must be capable of centrifuging microplates of 4.5 cm total height. Examples of suitable centrifuges, rotors, and adapters are given in Table 3. QIAGEN offers Centrifuge 4-15C or 4K15C — contact QIAGEN for details.
- DyeEx 96 plates must be centrifuged at  $1000 \times g$ . The appropriate speed can be calculated as follows:  $\text{rpm} = 1000 \times \sqrt{1000/1.12 r}$  ( $r$  = radius of rotor in mm).
- After centrifugation the gel-bed surface in the wells of the DyeEx 96 plate may vary due to the differing centrifugal force in the different wells (Figure 3). This is normal and has no effect on the performance of the DyeEx 96 procedure.
- Always use the waste collection plates provided with the DyeEx 96 Kit. These plates are also available separately (Collection Plates, 48-well; Cat. No. 19584). We do not recommend any other waste collection plates for use with the DyeEx 96 Kit.



**Figure 3.** Appearance of the DyeEx 96 plate after centrifugation. The gel-bed surfaces in the outer wells may be slanted due to the direction of the centrifugal forces.

# Standard Protocol

## Procedure for ABI PRISM 310, 3100, 373, 377, or MegaBACE 1000

1. **Take the DyeEx 96 plate out of the bag, and remove the tape sheets from the top and bottom of the DyeEx 96 plate.**

When handling the DyeEx 96 plate ensure that it remains horizontal. It is easier to remove the tape from the bottom first.

2. **Place the DyeEx 96 plate on the top of the collection plate (provided) and centrifuge for 3 min at the calculated speed.**

The collection plates are reusable. Discard the flow-through.

**Note:** Always use the waste collection plates provided with the DyeEx 96 Kit. These plates are also available separately (see ordering information, page 21). We do not recommend any other collection plates for use with the DyeEx Kit.

3. **Carefully place the DyeEx 96 plate on an appropriate elution plate with a suitable adapter.**

**Note:** The appropriate elution plate depends on the method of drying down the samples after elution (see Figure 4).

If the purified samples are to be dried on a thermal cycler, place the DyeEx 96 plate on a 96-well plate or on 12 x 8-well strips that are suitable for use with a thermal cycler and centrifuge with an appropriate adapter (Figure 4a). Suitable 96-well PCR plates include those from Corning/Costar (Cat. No. 6513) and suitable adapters include those from PE Biosystems (MicroAmp® Base, Cat. No. N801-0531).

If the purified samples are to be dried in a vacuum centrifuge, place the DyeEx 96 plate on a 96-well microplate (Figure 4b). Suitable microplates include those supplied by QIAGEN (Cat. No. 19581, see ordering information page 21).

**Note:** To ensure that the DyeEx 96 plate sits securely in the centrifuge rotor, the tops of the wells of the elution plate should be in direct contact with the base of the DyeEx 96 plate (see Figure 5, page 15).

4. **Slowly apply the sequencing samples in a volume of 10–20 µl to the gel bed of each well.**

**Note:** Pipet the sequencing reaction directly onto the center of the gel-bed surface (Figure 6, page 16). Do not allow the reaction mixture or the pipet tip to touch the sides of the wells. The samples should be pipetted slowly so that they are absorbed into the gel and do not flow down the sides of the gel bed. Avoid touching the gel-bed surface with the pipet tip.

**5. Centrifuge for 3 min at the calculated speed.**

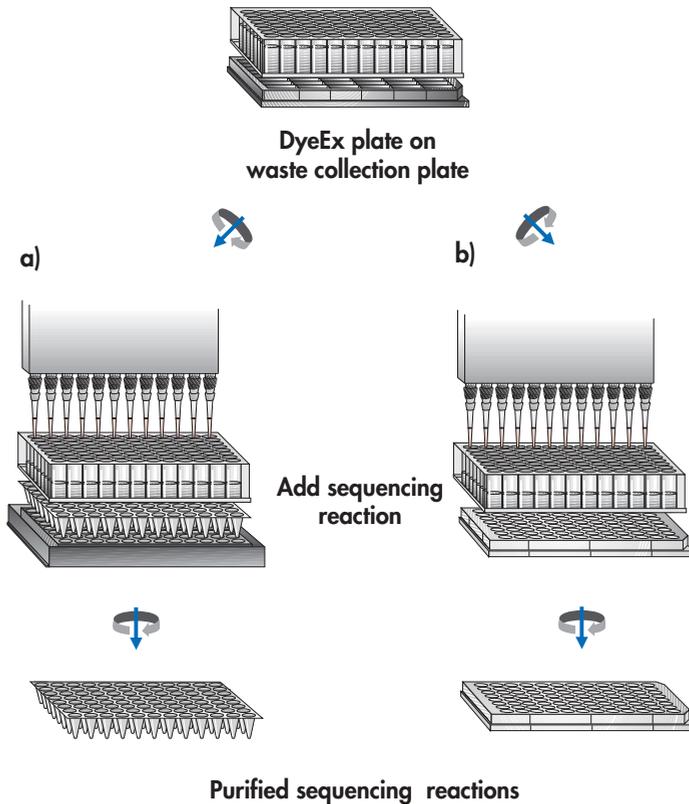
The eluate contains the purified sequencing reactions.

**Optional:** If using the MegaBACE 1000 or the ABI PRISM 3700 with a water loading protocol, it is possible to load the eluate directly onto the sequencer without drying down the sample. If using a formamide loading buffer, then proceed to step 6.

**6. Dry the samples and proceed according to the instructions provided with the DNA sequencer.**

Dry the samples in a vacuum centrifuge or uncovered at 70°C in a thermal cycler.

## DyeEx 96 Kit Procedure



**Figure 4.** DyeEx 96 procedure with elution into a plate suitable for drying in a thermal cycler (a) or vacuum centrifuge (b).

## Modified Protocol

### Procedure for ABI PRISM 3700 and Beckman CEQ 2000 for maximal signal intensities

**Note:** If maximal signal intensities are not required, the Standard Protocol can be used. To help decide which protocol to choose, see “Important Notes Before Starting”, on page 10.

1. **Take the DyeEx 96 plate out of the bag, and remove the tape sheets from the top and bottom of the DyeEx 96 plate.**

When handling the DyeEx 96 plate ensure that it remains horizontal. It is easier to remove the tape from the bottom first.

2. **Place the DyeEx 96 plate on the top of the collection plate (provided) and centrifuge for 1 min at the calculated speed.**

The collection plates are reusable. Discard the flow-through.

**Note:** Always use the waste collection plates provided with the DyeEx 96 Kit. These plates are also available separately (see ordering information, page 21). We do not recommend any other collection plates for use with the DyeEx 96 Kit.

3. **Place the DyeEx 96 plate on top of the collection plate, add 300  $\mu$ l water to each well, and centrifuge for 3 min at the calculated speed.**

We recommend the use of deionized water.

4. **Carefully place the DyeEx 96 plate on an appropriate elution plate with a suitable adapter.**

**Note:** The appropriate elution plate depends on the method of drying down the samples after elution (see Figure 4).

If the purified samples are to be dried on a thermal cycler, place the DyeEx 96 plate on a 96-well plate or on 12 x 8-well strips that are suitable for use with a thermal cycler and centrifuge with an appropriate adapter (Figure 4a). Suitable 96-well PCR plates include those from Corning/Costar (Cat. No. 6513) and suitable adapters include those from PE Biosystems (MicroAmp® Base, Cat. No. N801-0531).

If the purified samples are to be dried in a vacuum centrifuge, place the DyeEx 96 plate on a 96-well microplate (Figure 4b). Suitable microplates include those supplied by QIAGEN (Cat. No. 19581, see ordering information page 21).

**Note:** To ensure that the DyeEx 96 plate sits securely in the centrifuge rotor, the tops of the wells of the elution plate should be in direct contact with the base of the DyeEx 96 plate (see Figure 5).

5. **Slowly apply the sequencing samples in a volume of 10–20  $\mu$ l to the gel bed of each well.**

**Note:** Pipet the sequencing reaction directly onto the center of the gel-bed surface (Figure 6). Do not allow the reaction mixture or the pipet tip to touch the sides of the wells. The samples should be pipetted slowly so that they are absorbed into the gel and do not flow down the sides of the gel bed. Avoid touching the gel-bed surface with the pipet tip.

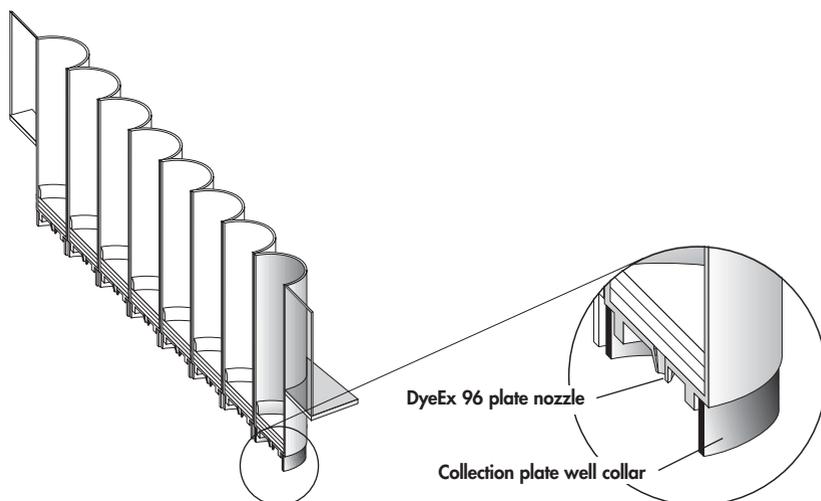
6. **Centrifuge for 3 min at the calculated speed.**

The eluate contains the purified sequencing reactions.

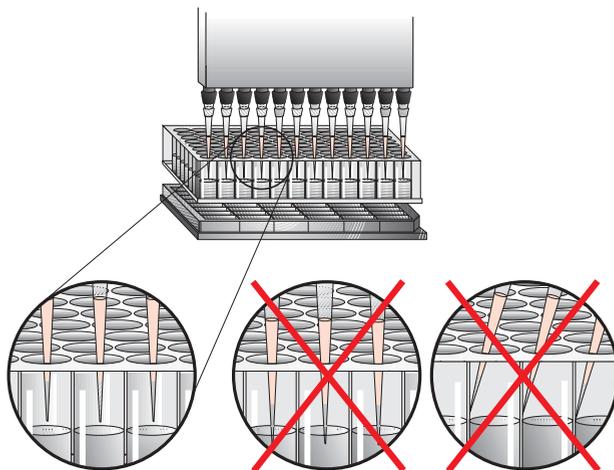
**Optional:** If using the ABI PRISM 3700 with the water loading protocol, it is possible to load the eluate directly onto the sequencer without drying down the sample. If using a formamide loading buffer, then proceed to step 7.

7. **Dry the samples and proceed according to the instructions provided with the DNA sequencer.**

Dry the samples in a vacuum centrifuge or uncovered at 70°C in a thermal cycler.



**Figure 5.** To ensure that the DyeEx 96 plate sits securely in the centrifuge rotor it must fit on the collection plate tightly. If the collection plate is suitable, the nozzles at the bottom of the DyeEx 96 plate protrude into the space inside the top of the collar of the collection plate wells as shown. A few types of collection plate are not suitable for use with DyeEx 96 plates.



**Figure 6.** Samples should be pipetted onto the center of the gel bed as shown in the closeup on the left.

# Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists at QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol(s) in this handbook or molecular biology applications (see last page for contact information).

---

## Comments and suggestions

---

### Dye-terminator blobs at the beginning of the sequencing profile (i.e., signals arising from unincorporated dye terminators)

- |                                |  |
|--------------------------------|--|
| a) Sample volume too high      | Ensure that the sample volume is 20 $\mu$ l. Sample of volumes >20 $\mu$ l can lead to dye-terminator carryover, causing blobs.  |
| b) Sample dispensed improperly | Pipet the sample directly onto the center of the gel-bed surface (Figure 2, see page 8 and Figure 6, above). Do not allow the reaction mixture or the pipet tip to contact the sides of the gel-bed or the sides of the DyeEx 2.0 spin columns or wells of the DyeEx plates. |
| c) Sample applied too fast     | Pipet the sample slowly so that the drops are absorbed into the gel and do not flow down the sides of the gel bed.   |

### Low signal intensity

- a) Sample volume too low

#### DyeEx 2.0 Spin Kit

Ensure that the applied sample volume is  $\geq 10$   $\mu$ l. If necessary adjust the sample volume to 20  $\mu$ l using distilled water prior to loading onto the DyeEx 2.0 spin column. This provides easier handling, greater reproducibility, and minimized error.

#### DyeEx 96 Kit

Ensure that the sample volume is  $\geq 10$   $\mu$ l. If necessary, adjust the volume of the samples to 20  $\mu$ l with distilled water prior to loading onto the DyeEx 96 plate. Adjusting the volume to 20  $\mu$ l leads to an increase of signal intensity of up to 30%.

### **Gel-bed heights in DyeEx 96 plate different after centrifugation**

- a) Surfaces of the gel beds appear different

After centrifugation the gel-bed surface in the wells of the DyeEx 96 plate may vary due to the differing centrifugal force in the different wells (Figure 3). This is normal and has no effect on the performance of the DyeEx 96 procedure.

Always use the waste collection plates provided with the DyeEx 96 Kit. These plates are also available separately (Collection Plates, 48-well; Cat. No. 19584). We do not recommend any other collection plates for use with the DyeEx 96 Kit.

# Appendix

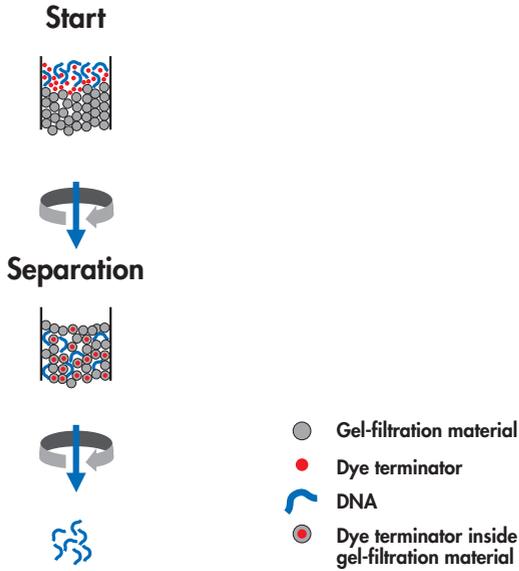
## DyeEx principle

The DyeEx procedure combines the convenience of a ready-to-use spin-column or 96-well plate format with the effective separation properties of gel-filtration chromatography. Gel-filtration chromatography separates molecules based on molecular weight. DyeEx Kits use gel-filtration material consisting of spheres with uniform pores. When sequencing reactions are applied onto DyeEx modules, dye terminators diffuse into the pores and are retained in the gel-filtration material, while the DNA fragments are excluded and recovered in the flow-through (Figure 7, see page 19).

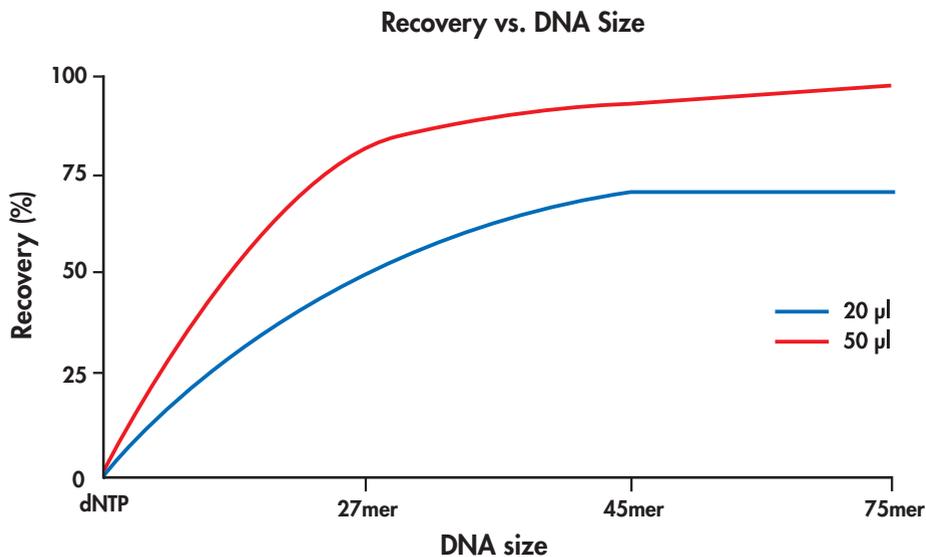
The separation efficiency depends mainly on two parameters — DNA size and sample volume. For any given sample volume, the DNA recovery increases with increasing DNA size (Figure 8, see page 20). However, a large sample volume increases not only the recovery of DNA fragments but also the level of contamination with dye terminators and nucleotides (Figure 9, see page 20). A small sample volume gives low contamination with dye terminators but also reduces the signal intensity and read length from the cleaned up DNA upon sequencing. Sample volumes of 10–20  $\mu\text{l}$  are optimal for efficient cleanup of dye-terminator sequencing reactions using the DyeEx procedure.

## Special application for labeling-reaction cleanup

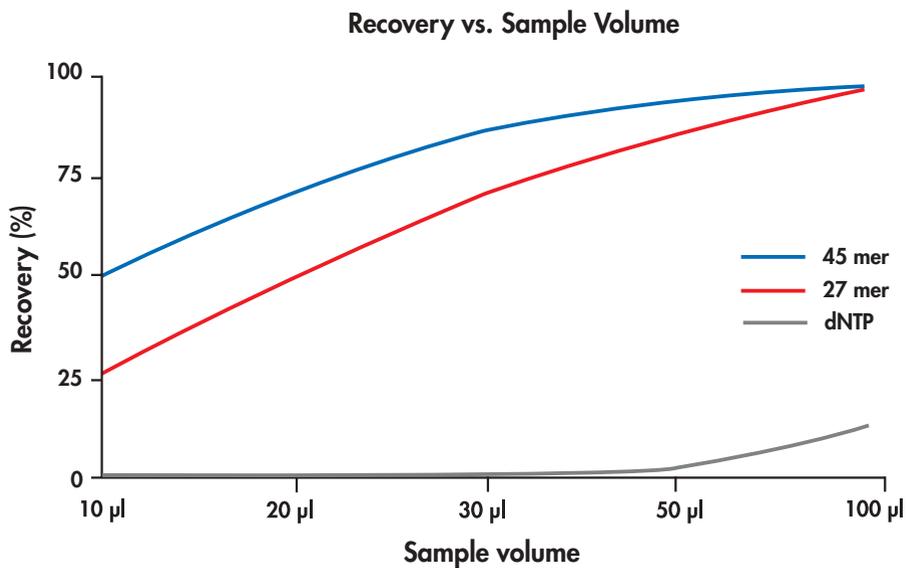
DyeEx Kits have been developed for cleanup of dye-terminator sequencing reactions. However, they can also be used for removal of unincorporated nucleotides from radioactively labeled DNA fragments using the protocols in this handbook. The removal of nucleotides depends on the sample volume. Typically more than 99% of nucleotides are removed from sample volumes  $\leq 50 \mu\text{l}$ . See Figure 9 (page 20) for the effect of sample volume on recovery.



*Figure 7. DyeEx separation principle.*



**Figure 8.** Effect of DNA size on recovery. 1 µg of oligomer was purified according to the DyeEx protocol.



**Figure 9.** Effect of sample volume on recovery. 1 µg of oligomer was purified according to the protocol.

## Ordering Information

Product	Contents	Cat. No.
DyeEx 2.0 Spin Kit (50)	50 DyeEx 2.0 Spin Columns, Collection Tubes (2 ml)	63204
DyeEx 2.0 Spin Kit (250)	250 DyeEx 2.0 Spin Columns, Collection Tubes (2 ml)	63206
DyeEx 96 Kit (4)	4 DyeEx 96 Plates; 4 Collection Plates, 48-well	63181
DyeEx 96 Kit (24)	24 DyeEx 96 Plates; 4 Collection Plates, 48-well	63183
Collection Plates, 48-well (24)	48-well waste collection plates, 24 per case	19584
96-Well Microplates RB (24)	96-well microplates with round-bottom wells plus lids, 24 per case	19581

### Trademarks

Patent or patent-pending technology and/or registered or registration-pending trademarks of QIAGEN: QIAGEN®, DyeEx™.

ABI PRISM and MicroAmp are registered trademarks of Applied Biosystems Corporation or its subsidiaries.

BigDye is a trademark of Perkin-Elmer Applied Biosystems Corporation or its subsidiaries.

CEQ is a trademark of Beckman Coulter, Inc.

MegaBACE is a trademark of Amersham Pharmacia Biotech

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

© 2002 QIAGEN, all rights reserved.

# QIAGEN Companies



Please see the inside front cover for contact information for your local QIAGEN office.

## QIAGEN Distributors

### Argentina

Tecnolab S.A.  
Charlone 144 - C 1427BXD  
Capital Federal  
Tel: (011) 4555 0010  
Fax: (011) 4553 3331  
E-mail: info@tecnolab.com.ar  
Web site: www.tecnolab.com.ar

### Austria/Slovenia

VWR International GmbH  
Zimbaggasse 5  
1147 Wien  
Austria  
Tel: (01) 576 00 0  
Fax: (01) 576 00 350  
E-mail: merck-wien@merckeurolab.at  
Web site: www.vwr.com

### Belgium/Luxemburg

Westburg b.v.  
P.O. Box 214  
3830 AE Leusden  
The Netherlands  
Tel: 0800-19815  
Fax: (31) 33-4951222  
E-mail: info@westburg.nl  
Web site: www.westburg.nl

### Brazil

Uniscience do Brasil  
Av. Cândido Portinari, 933/937  
05114-001 São Paulo - SP  
Brazil  
Tel: 011 3622 2320  
Fax: 011 3622 2323  
E-mail: info@uniscience.com  
Web site: www.uniscience.com

### China

Gene Company Limited  
Unit A, 8/F., Shell Industrial Building  
12 Lee Chung Street  
Chai Wan, Hong Kong, P.R.C.  
Tel: (852)2896-6283  
Fax: (852)2515-9371  
E-mail: info@genehk.com  
Beijing: gene@public2.bta.net.cn  
Shanghai: gene@public.sta.net.cn  
Chengdu: gene@public.cd.sc.cn  
Guangzhou: gzyitao@public.guangzhou.gd.cn

### Cyprus

Sciennomics Ltd  
34, Zenonos Sozou Str.  
1075 Lefkosta  
Tel: 02-765 416  
Fax: 02-764 614  
E-mail: sarpetso@spidernet.com.cy

### Czech Republic

BIOCONSULT spol. s.r.o.  
Božajoviců 145  
142 01 Praha-Libuš  
Tel/Fax: (420) 2 417 29 792  
E-mail: bio-cons@login.cz  
Web site: www.bio-consult.cz

### Denmark

VWR International ApS  
Roskildevej 16  
2620 Albertslund  
Tel: 43 86 87 88  
Fax: 43 86 87 90  
E-mail: info@dk.vwr.com  
Web site: www.vwr.com

### Egypt

Clinilab  
P.O. Box 12 ElManial  
4, 160 St., ElEtahed Square  
Riham Tower, ElMaadi  
Cairo  
Tel: 52 57 212  
Fax: 52 57 210  
E-mail: Clinilab@link.net

### Finland

VWR International Oy  
Niityrinne 7  
02270 Espoo  
Tel: (09) 804 551  
Fax: (09) 8045 5200  
E-mail: info@fi.vwr.com  
Web site: www.vwr.com

### France

BioAnalytica S.A.  
11, Laskareos Str.  
11471 Athens  
Tel: (10)640 03 18  
Fax: (10)646 27 48  
E-mail: bioanalyt@hol.gr

### India

Genetix  
C-88, Kirti Nagar  
Lower Ground Floor  
New Delhi-110 015  
Tel: (011)542 1714  
Fax: or (011)515 9346  
(011)546 7637  
E-mail: genetix@nda.vsnl.net.in

### Israel

Westburg [Israel] Ltd.  
1, Habursekai St. Kiryat Ha'asakim  
Beer Sheva 84899  
Tel: 08-6650813/4  
or 1-800 20 22 20 (toll free)  
Fax: 08-6650934  
E-mail: info@westburg.co.il  
Web site: www.westburg.co.il

### Korea

IRS Laboratories, Inc.  
Seongbuk P.O. Box 61  
Seoul, 136-600  
Tel: (02) 924-86 97  
Fax: (02) 924-86 96  
E-mail: webmaster@lrslab.co.kr  
Web site: www.lrslab.co.kr

### Malaysia

RESEARCH BIOLABS SDN. BHD.  
11-A, Jalan BK 5A/2  
Bandar Kinrara  
47100 Puchong, Selangor Darul  
Ehsan  
Tel: (603)-8070 3101  
Fax: (603)-8070 5101  
E-mail: biolabs@tm.net.my  
Web site: www.researchbiolabs.com

### Mexico

Quimica Valaner S.A. de C.V.  
Jalapa 77, Col Roma  
Mexico D.F. 06700  
Tel: (55) 55 25 57 25  
Fax: (55) 55 25 56 25  
E-mail: qvalaner@infosel.net.mx

### The Netherlands

Westburg b.v.  
P.O. Box 214  
3830 AE Leusden  
Tel: (033)-4950094  
Fax: (033)-4951222  
E-mail: info@westburg.nl  
Web site: www.westburg.nl

### New Zealand

Biolab Scientific Ltd.  
244 Bush Road  
Albany, Auckland  
Tel: (09)980 6700  
or (080)933 966  
Fax: (09)980 6788  
E-mail: info@biolab.co.nz  
Web site: www.biolab.co.nz

### Norway

VWR International AS  
Kakkelovnskroken 1  
P.B. 45, Kalbakken,  
0901 Oslo  
Tel: 22 90 00 00  
Fax: 22 90 00 40  
E-mail: info@no.vwr.com  
Web site: www.vwr.com

### Poland

Syngen Biotech Sp.z.o.o.  
ul.Legnicka 62 A  
54-204 Wrocław  
Tel: (071) 351 41 06  
or 0601 70 60 07  
Fax: (071) 351 04 88  
E-mail: info@syngen.com.pl  
Web site: www.syngen.com.pl

### Portugal

IZASA PORTUGAL, LDA  
Rua do Proletariado, 1 - Quinta do  
Paizinho  
2795-648 Carnaxide  
Tel: (21) 424 7312  
Fax: (21) 417 2674

### Singapore

Research Biolabs Pte Ltd  
211 Henderson Road #1401  
Henderson Industrial Estate  
Singapore 159552  
Tel: 2731066  
Fax: 2734914  
E-mail: biolabs@singnet.com.sg

### Slovak Republic

BIOCONSULT Slovakia spol. s.r.o.  
Ružová dolina 6  
SK-821 08 Bratislava 2  
Tel/Fax: (02) 5022 1336  
E-mail: bio-cons@post.sk  
Web site: www.bio-consult.cz

### South Africa

Southern Cross Biotechnology (Pty)  
Ltd  
P.O. Box 23681  
Claremont 7735  
Cape Town  
Tel: (021) 671 5166  
Fax: (021) 671 7734  
E-mail: info@scb.co.za  
Web site: www.scb.co.za

### Spain

IZASA, S.A.  
Aragón, 90  
08015 Barcelona  
Tel: (93) 902.20.30.90  
Fax: (93) 902.22.33.66  
E-mail: suministros@izasa.es

### Sweden

VWR International AB  
Fagerstagatan 18A  
163 94 Stockholm  
Tel: (08) 621 34 00  
Fax: (08) 760 45 20  
E-mail: info@se.vwr.com  
Web site: www.vwr.com

### Taiwan

TAIGEN Bioscience Corporation  
3F, No. 306, Section 4  
Chen-Der Road  
111 Taipei  
Taiwan, R.O.C.  
Tel: (02) 2880 2913  
Fax: (02) 2880 2916  
E-mail: taigen@ms10.hinet.net

### Thailand

Theera Trading Co. Ltd.  
64 Charan Sanit Wong Road  
(Charan 13) Bangkokyay  
Bangkok 10600  
Tel: (02) 412-5672  
Fax: (02) 412-3244  
E-mail: theetrade@samart.co.th

## QIAGEN Importers

### Central & South America

(except Argentina and Brazil)  
Labtrade Inc.  
6157 NW 167th Street F-26  
Miami, FL 33015  
USA  
Tel: (305) 828-3818  
Fax: (305) 828-3819  
E-mail: labtrade@icannet.net  
Web site: www.labtrade.com

### Hungary

Kasztel-Med Co. Ltd.  
Kelenhegyi út 22  
H-1118  
Tel: (01) 385 3887  
Fax: (01) 3810695  
E-mail: info@kasztel.hu  
Web site: kasztel.hu

### Estonia

PEAI:Est OÜ  
Riia 185  
51014 Tartu  
Tel: (051) 65 830  
Fax: (07) 383 360  
E-mail: langel@ut.ee

### Saudi Arabia

Abdulla Fouad Co. Ltd.  
Medical Supplies Division  
Prince Mohammed Street  
P.O. Box 257, Dammam 31411  
Kingdom of Saudia Arabia  
Tel: (03) 8324400  
Fax: (03) 8346174  
E-mail: sadiq.umar@abdulla-fouad.com

### Turkey

Medek Medikal Ürünler  
ve Sağlık Hizmetleri A.Ş.  
Bagdat Cad. 449 D.9 Suadiye  
81070 Istanbul  
Tel: (216) 302 15 80  
Fax: (216) 302 15 88  
E-mail: aktalp@turk.net

### All other countries

QIAGEN GmbH, Germany

