

DyeEx™ Handbook

For

NEW DyeEx 2.0 Spin Kit
DyeEx 96 Kit

May 2002



QIAGEN Worldwide

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Kit Contents

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

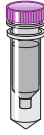
Table 1. DyeEx Kit specifications

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

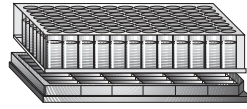
* We recommend the DyeEx 96 Kit using the optimized protocol for the ABI PRISM 3700 sequencer and CEQ 2000.

DyeEx 2.0 Spin Kit Procedure

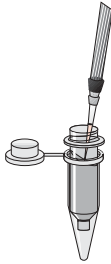
DyeEx 96 Kit Procedure



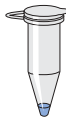
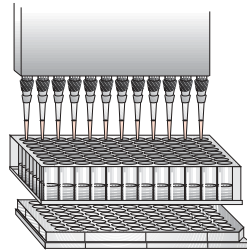
DyeEx 2.0 spin column
in collection tube



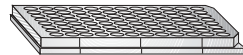
DyeEx plate on waste
collection plate



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

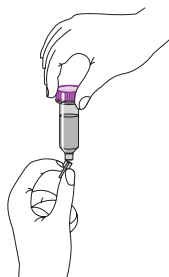


Figure 1. Snapping off the bottom closure of the DyeEx 2.0 spin column (do not screw).

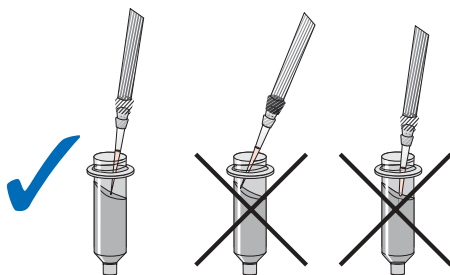


Figure 2. Instructions for sample application to the DyeEx 2.0 spin column.

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 μ 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 μ l. For easier handling, more reproducible pipetting, and reduced error with sample volumes <10 μ l, we recommend adjusting the volume to 20 μ 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 [†]	PTS-2000	Included	2300 rpm
Eppendorf 5810 [†]	MTP Rotor A-4-62	4 MTP adapters	2400 rpm
Heraeus Megafuge 1.0 [†]	Cat. No. 75002704	Microplate carrier (Cat. No. 75007586)	2500 rpm
Heraeus Megafuge 2.0 [†]	Cat. No. 75008155	Microplate carrier (Cat. No. 75008083)	2400 rpm
Hettich ROTANTA 96 or 46 [†]	Cat. No. 4444	4 MTP adapters	2300 rpm
Hettich ROTIXA 120 or 150S [†]	Cat. No. 4294	4 MTP adapters	2300 rpm
Sorvall T-6000 [†]	H-1000B	Microplate Carrier	2450 rpm
Sorvall RT7 [†]	RTH-250	Microplate Carrier	2450 rpm
Centra CL3/CLR [†]	Rotor 244	Included	4000 rpm
Multi/Multi RF [†]	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.

[†] 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.

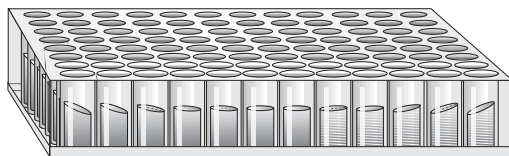


Figure 3. Appearance of the DyeEx 96 plate after centrifugation. The gel-bed surfaces in the outer wells may be slanted due to the direction of the centrifugal forces.

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

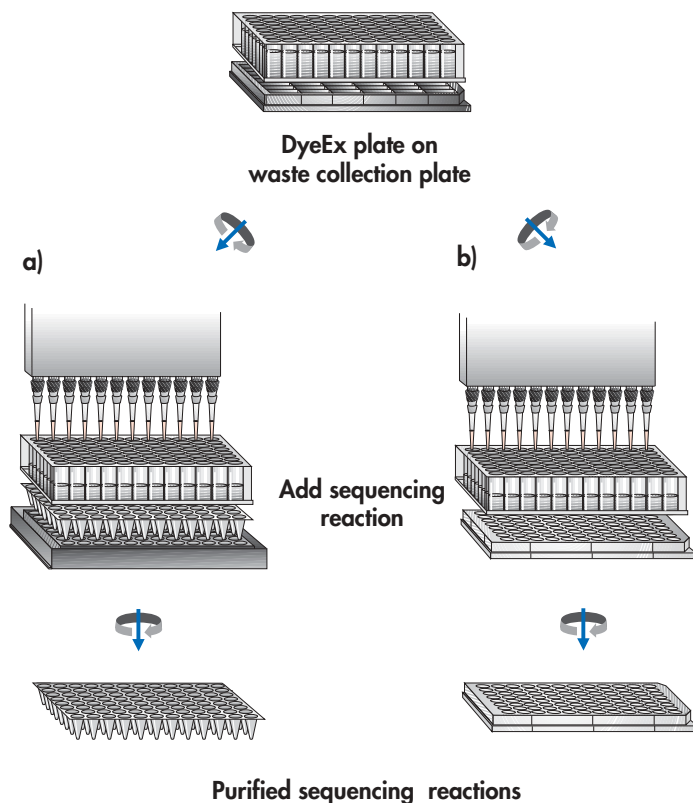


Figure 4. DyeEx 96 procedure with elution into a plate suitable for drying in a thermal cycler (a) or vacuum centrifuge (b).

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 μ 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 μ 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.

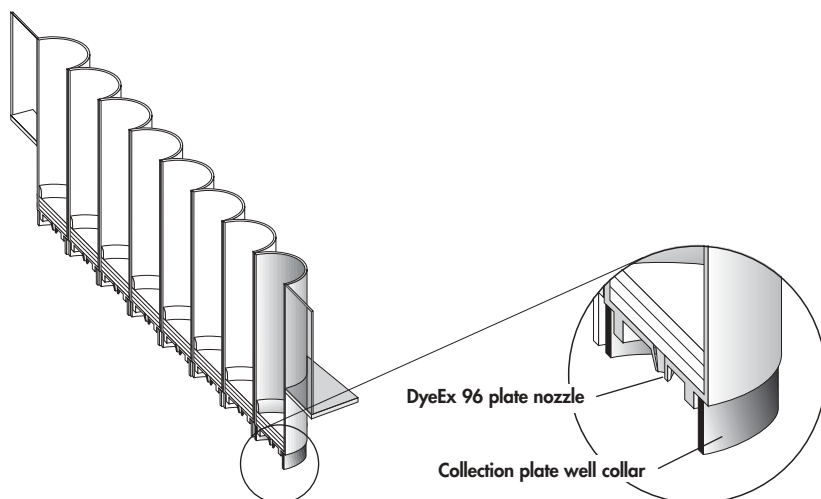


Figure 5. To ensure that the DyeEx 96 plate sits securely in the centrifuge rotor it must fit on the collection plate tightly. If the collection plate is suitable, the nozzles at the bottom of the DyeEx 96 plate protrude into the space inside the top of the collar of the collection plate wells as shown.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

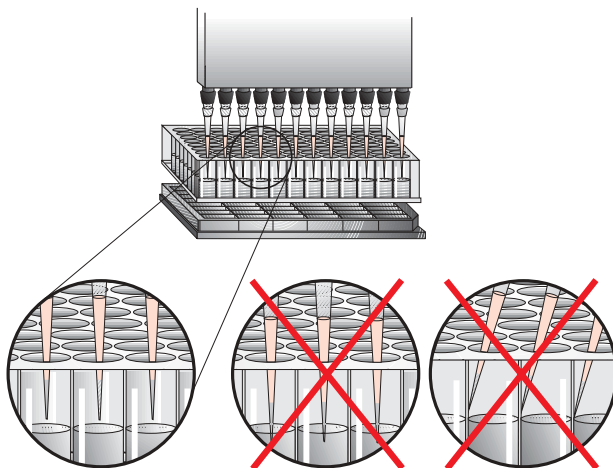


Figure 6. Samples should be pipetted onto the center of the gel bed as shown in the closeup on the left.

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 μ l. Sample of volumes >20 μ 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 ≥ 10 μ l. If necessary adjust the sample volume to 20 μ 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 ≥ 10 μ l. If necessary, adjust the volume of the samples to 20 μ l with distilled water prior to loading onto the DyeEx 96 plate. Adjusting the volume to 20 μ 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 μl 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.

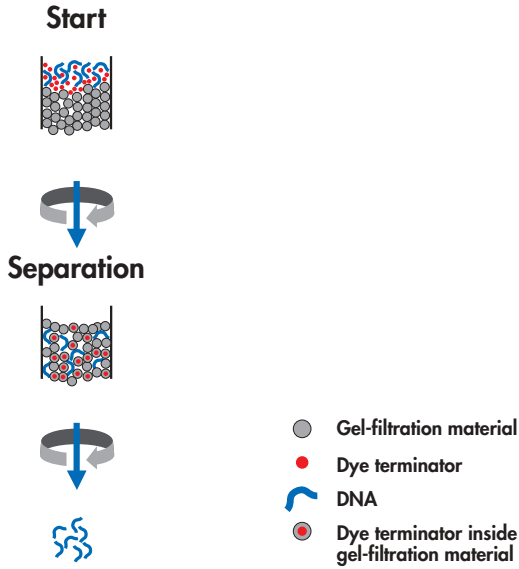


Figure 7. DyeEx separation principle.

Recovery vs. DNA Size

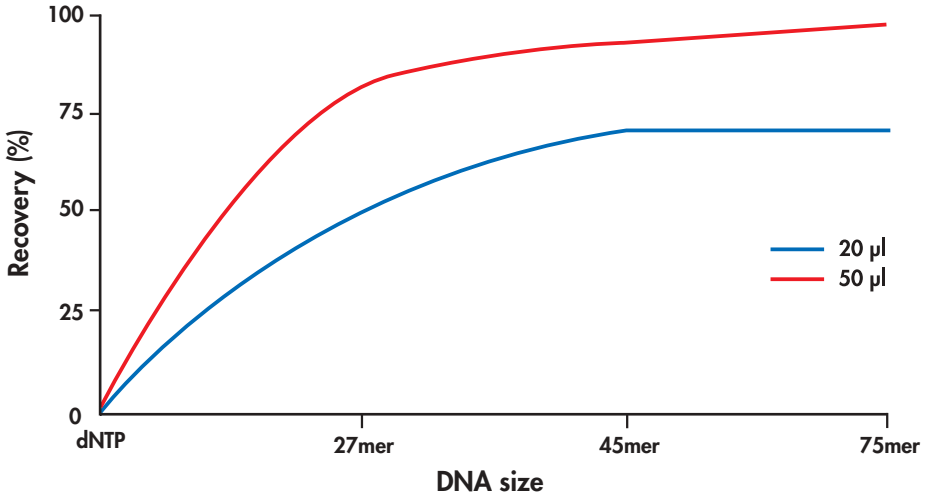


Figure 8. Effect of DNA size on recovery. 1 µg of oligomer was purified according to the DyeEx protocol.

Recovery vs. Sample Volume

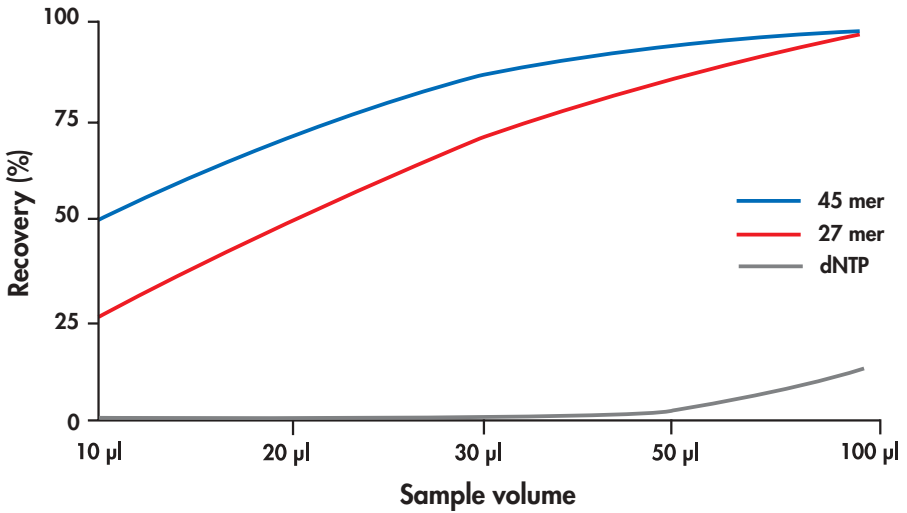


Figure 9. Effect of sample volume on recovery. 1 µg of oligomer was purified according to the protocol.

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

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