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Introduction

Excess unincorporated, nonradioactive label can cause high background fluorescence in automated sequencing gels. For optimal sequencing results, remaining labeled dideoxynucleotides should be removed prior to electrophoresis. The Ultra-Sep™ Dye-Removal Kit is designed for effective and reliable removal of unincorporated terminators from sequencing reactions.

In addition, the Ultra-Sep™ Dye-Removal Kit can also be used for many other applications:

- removal of salts and unwanted low-molecular-weight impurities
- Purification of fluorescent reaction mixtures
- Removal of free and labeled dNTP's from DNA or RNA, as in:
  1. nick translation
  2. end-labeling reactions
  3. polymerization reaction
- Purification and desalting of proteins
- Removal of excess nucleotides and small primers in PCR reactions
- Removal of radioactive precursors
- Desalting and buffer exchange

Principle

Generally, several methods can be used to purify DNA from labeling reactions: precipitation, silica-resin binding, gel filtration. Among those methods, gel filtration is the most efficient way to purify DNA from small volume of reaction mixture without losing DNA. The Ultra-Sep gel filtration material consists of spheres with uniform pores and separates molecules according molecular wight. The gel will provide excellent recovery of DNA fragments **>16 base pairs or 25-mer** while removing **98%** of salts, NTP's and other low molecular weight components. Simply apply the sequencing reaction into the Ultra-Sep™ Dye-removal Column, the dye terminators diffuse into the pores and are retained in the gel filtration material, while the labeled DNA fragments are excluded and recovered in the flow-though.

Ultra-Sep™ Dye-Removal kit is optimized for cleaning up sequencing reactions containing any type of dye terminators, such as dRhodamine, Rhodamine. This kit works well with the Tag Dyedeoxy™ and ABI Prsim™ terminator cycle sequencing kits. By combining gel filtration with micro spin technology, this Ultra-Sep™ Dye-Removal kit can clean up a sequencing reaction within 7-10 minutes.

Kit Components

Product Number	S5912-00A (5 Preps)	S5912-01A (50 Preps)	S5912-02A (200 Preps)
Ultra-Sep™ Dye-Removal Column	5	50	200
2 mL Collection Tube	5	50	200
User Instruction Manual	1	1	1

Storage and Stability

All components of the Ultra-Sep™ Dye removal kits are stable for at least 12 months from the date of purchase when stored at 15°C-25°C. For long term storage, this kit can be stored at 8°C-15°C. Contents of the kit should not be refrigerated at any time.

Additional Materials Supplied by User

- Microcentrifuge (Eppendoff 5415C,5417C, Variable Speed or equivalent)
- Variable pipets (Pipetman 100 µl)
- Pipet Tips
- Pipet Bulb
- Sterile 1.5 ml microcentrifuge tube
- Microtube Rack
- Reagent grade water or desired buffer

Before Starting

- Centrifugation- All centrifuge steps should be performed at 735 x g. It is recommended to use horizontal or swinging-bucket rotor because they will give maximum yield and efficiency. However, fixed-angle-rotor micocentrifuges provide acceptable performance and save time.
- Allow refrigerated columns to warm to room temperature before continuing the dye removing procedure (For pre-reconstituted column).
- Centrifuge tubes - Use 1.5 ml sterile microcentrifuge tubes.
- Before using a Ultra-Spin™ column, it is important to calculate the speed at which the column should be centrifuged. The appropriate speed can be calculated from following formula:

$$RCF = (1.12)\ (r)\ (rpm/1000)^2$$

where RCF = relative centrifugal force; r = radius in mm measured from center of spindle to bottom of rotor bucket; and rpm = revolutions per minutes.

For a force of 735 x g, the above equation resolves to:

$$rpm = (1000)\ (657/r)^{½}$$

For example, with a microcentrifuge rotor having a radius of 73 mm, the appropriate speed would be 3000rpm. For a clinical centrifuge with a rotor having a radius of 110 mm, the appropriate speed would be 2444 rpm. See Table 2 for information on available microcentifuges.

Table 2. Microcentrifuge speed setting needed to achieve centrifugal forces of 735 x g

Microcentrifuge	Rotor capacity	Rotor radius	Approximate speed (rpm)
Eppendorf 5415C	18	73mm	3000
Eppendorf 5402	18	73mm	3000
Eppendorf 5417C	30	92mm	2700
Biofuge 13	24	79mm	2900
RX-200 No.20N	24	98mm	2600
MCX-150 TMA-11	18	73mm	3000
Micro-cooling RA-155	24	59mm	3300
SCT 15B RT15A	12	70.6mm	3100

Procedure

The following protocol suitable for sequencing analysis using ABI Prism 310, 370, 373 A and 377A sequencers.

1. **Gently vortex the spin column to resuspend the resin and open the column cap by a half turn, then snap off the bottom closure.**

2. **Place the column into a new 2 ml collection tube.**

3. Centrifuge 2-3 minutes at appropriate speed (735 x g).

   Note: It is important to keep track of the position of the column using the orientation mark molded into the column. If there is drop at the end of column, blot it dry. Do not allow the resin material dry excessively. Process the sample within the next few minutes.

4. **Carefully transfer the spin column to a clean 1.5 ml microcentrifuge tube.**

5. **Hold the column up to the light. Transfer 15-20µl of completed sequencing reaction mixture to the top of the gel.**

- Carefully dispense the samples directly onto the center of the gel bed at the top of the column, without disturbing the gel surface.

- Do not contact the sides of the column with the reaction mixture or the sample pipet tip, since this can reduce the efficiency of the purification.

- This protocol is suitable for the sequencing reactions with volume 10-20µl. For reaction volume less than 10µl, adjust the total volume to 20µl by adding distilled water.

6. **Centrifuge 2-3 minutes at the calculated speed.**

   Note: Maintain proper column orientation before centrifugation can improve the reproducibility. The highest point of the gel media in the column should be always point toward the outside of the rotor.

7. **Remove the spin column from the micocentrifuge tube. The microcentrifuge tube contains the purified the DNA.**

8. **Dry the sample in a vacuum centrifuge. Do not apply heat. Proceed according the instructions provided with the DNA sequencer.**

Trouble shooting

Problem	Likely Cause	Suggestions
Dye terminator remain in the eluted DNA and cause blobs.	1. Sample volume is large than 20µl and it cause dye terminator carry over,	Ensure that the sample volume is 20µl.
	2.Sample dispensed improperly	1.Make sure that the sample are loaded onto the center of the gel bed surface. 2.Do not touch the side of the column during sample application. 3. Apply the sample slowly onto the gel bed.
Low signal intensity	Sample volume too low	ensure that the sample volume is more than 10µl, adjust the volume to 20µl if necessary
	Column is not completely dried before load the sample.	Make sure to remove the last drop at the end of the column before load the sample.

Product No.	Product Name	Description
D6493-01/02 D6492-01/02	Cycle-Pure Kit	PCR product purification, Q-Column format & V-column format.
D1043-01/02	E-Z 96 Cycle-Pure Kit	96 well format PCR purification
D6537-01/02 D6538-01/02	DNA Probe Purification Kit	DNA probe purification, Q-column & V-column format
D2561-01/02	Poly-Gel DNA Purification Kit	Isolate DNA from polyacrylamide gel
D2501-01/02 D2500-01/02	Gel Extraction Kit	Agarose gel extraction using spin column technology
D2510-01/02	Ultra-Sep Gel Purification Kit	Agarose gel extraction using silica beads.
R6376-01/02	Poly-Gel RNA Purification Kit	Isolate RNA from polyacrylamide gel
R6537-01/02 R6538-01/02	RNA Probe Purification Kit	RNA probe purification, Q-column & V-column format

***** All OBI products available with size if 50 preps and 200 preps. Product number end with"-01" represent 50 preps kit and "-02" represent 200 preps kit.

To place order or have technical question, please call us @ 800.832.8896 or fax: 888.624.1688. You also can contact us from our web site: www.omegabiotek.com

Ordering Information