

Dynabeads® Sequencing Clean-Up

Catalog nos. 66101, 66102

Store at 2 °C to 8 °C

Rev. Date: June 2012 (Rev. 004)

Product Contents

Cat. no.	Volume
66101	1 mL
66102	5 × 1 mL

Product capacity:

66101: 500 samples

66102: 2500 samples

Dynabeads® Sequencing Clean-Up contains ~14.4 mg beads/mL supplied in purified water and tetraethylglycol.

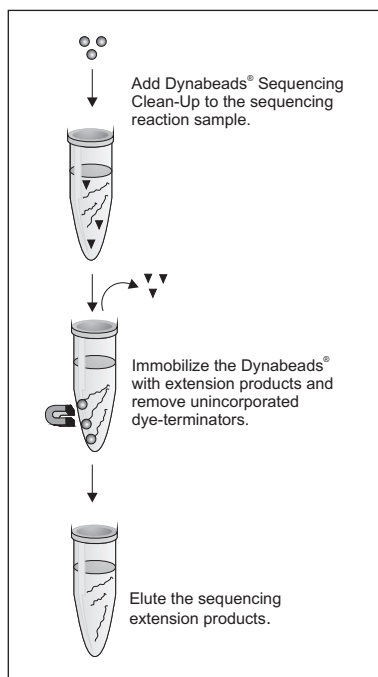


Figure 1: Outline of the purification procedure. Mixing Dynabeads® with the sequencing reaction sample cause the extension products to bind to the beads. A magnet is used to separate out the beads with bound extension products, and to further wash the complex. Before use in capillary electrophoresis, elute the pure extension products using high purity water.

Product Description

Dynabeads® Sequencing Clean-Up is designed for simple and rapid purification of dye-terminator sequencing reactions, leaving excess dye terminators, enzymes, and salt behind. Purified sequencing extension products are eluted in pure water and ready for injection in capillary sequencing systems.

Dynabeads® Sequencing Clean-Up is based on the unique Dynabeads® biomagnetic separation technology, giving fast and easy isolation, reproducible results, and eliminating the use of centrifugation and filtration. This allows easy automation of the protocol.

Dynabeads® Sequencing Clean-Up removes any components from dye-terminator sequencing reactions that can disturb the results of the following analysis by capillary electrophoresis.

This gives highly pure extension products, leading to optimal sequencing conditions and sequencing results with long reading lengths.

Dynabeads® are added to the sequencing reaction sample. The special binding buffer enhances the binding of extension products to the beads, leaving unwanted components free in solution (fig. 1).

Using a magnet, the beads with bound extension products can be pulled to the side of the tube, allowing easy removal of supernatant containing dye terminators, enzymes, and salt.

Similarly, washing is easily performed using freshly made 85% ethanol as washing solution. In the final step, the pure extension products are eluted from the beads by adding highly purified water, and the magnet is used to remove the beads from the eluate. The pure extension products are now ready for use in capillary or gel-based sequencing.

Required Materials

- Magnet (DynaMag™ portfolio). See www.lifetechnologies.com/magnets for recommendations.
- Test tubes/microtiter plates and pipettes.

General Guidelines

- First time using a new bottle, add 9.0 mL 96% ethanol to Dynabeads® Sequencing Clean-Up and thoroughly mix to a homogenous suspension (Dynabeads® Solution). After ethanol addition, mark the bottle with date for addition.
- Always use freshly made 85% ethanol for washing. When scaling the protocol to accommodate for other sample volumes, always keep a 1:2 ratio of sample: Dynabeads®.
- We recommend to aspirate 25 µL of the total 30 µL used for elution (step 10 in the "Protocol") ensure that no beads are transferred together with the purified extension products.

Protocol

1. Transfer 10 µL sequencing reaction sample to a new 1.5-mL microcentrifuge tube.
2. Add 20 µL Dynabeads® Solution (see "General Guidelines") to the tube. Mix thoroughly by pipetting.
3. Incubate at room temperature for 15 min.
Note: The incubation time may be reduced to 2 min if the signal is sufficient.
4. Place the tube on a magnet for 1 min. Discard supernatant.
5. Add 30 µL of 85% ethanol to the Dynabeads® with bound extension products and mix. Place the tube on a magnet and discard supernatant.
6. Air-dry sample for 5 min.
7. Add 30 µL highly purified water.
8. Mix by pipetting until beads are resuspended.
9. Incubate 2 min at room temperature.
10. Place the tube on a magnet for 1 min. Transfer 25 µL of eluted extension products in water to plate/tube used for sequencing.

Troubleshooting Guide

Problem	Suggested Solution
Signal strength is above recommended value for the sequencing system used.	<ul style="list-style-type: none">• Reduce incubation time (Protocol, step 3).• Reduce injection time.
Signal strength is below recommended value for the sequencing system used.	<ul style="list-style-type: none">• Increase injection time.• May indicate insufficient ethanol concentration in the Dynabeads® Solution or Wash Solution. Make fresh ethanol-diluted Dynabeads® Solution and/or re-fill the reservoir (automated systems) with fresh Dynabeads® Solution.• Replace the wash solution with fresh 85% ethanol.• Increase elution time (Protocol, step 9).
"Dye blob" appears in the beginning of the electropherogram, followed by weak or absent signal.	<ul style="list-style-type: none">• May indicate incomplete removal of supernatant after binding (Protocol, step 4). Assure complete removal of supernatant. Adjust pipetting height in automated systems.• Excessively vigorous mixing may cause droplets to attach to the side of the tube/well, and these are not removed by pipetting. To avoid this, mix less violently or spin the tube prior to magnetic separation.
Weak signals appear in the beginning of the electropherogram.	<ul style="list-style-type: none">• May indicate insufficient ethanol concentration in the Dynabeads® Solution. Make fresh ethanol-diluted Dynabeads® Solution and/or refill the reservoir (automated systems) with fresh Dynabeads® Solution.• Replace the Wash Solution with fresh 85% ethanol.
"Blob" appears near base position 100-150 in the electropherogram.	<ul style="list-style-type: none">• May be caused by residual ethanol in the eluate. Increase drying time (Protocol, step 6), or dry the samples under vacuum or with gentle heating.

Description of Materials

Dynabeads® are uniform, superparamagnetic, polymer beads (1 µm in diameter). Dynabeads® with affinity for sequencing extension products are supplied in a special binding buffer giving optimal conditions for binding, purification, and elution of highly pure extension products. The suspension is ready to use after addition of 96% ethanol.

Related Products

Product	Cat. no.
DynaMag™-2	12321D
DynaMag™-5	12303D
DynaMag™-15	12301D

REF on labels is the symbol for catalog number.

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