CleaniBeads™

User Manual

Solid Phase Reversible Immobilization (SPRI)
Magnetic bead-based DNA purification

For Catalog Numbers: CB-2ML, CB-10ML, CB-25ML, CB-50ML

Version 1.0 / August 2018

Contents

1. Product Description

1.1 Introduction

CleaniBeads are magnetic particles prepared in a well-optimised solution, tailor-made for efficient and fast purification of DNA molecules of size larger than 100 bp. The protocol involves no labour-intensive centrifugation or filtration steps and is readily automatable.

CleaniBeads can be used to purify amplicons/DNA after PCR or other enzymatic reactions. The method will effectively remove unused oligos, salts, enzymes and dNTPs, yielding high quality DNA for downstream applications such as DNA sequencing, cloning and genotyping.

1.2 Storage conditions

CleaniBeads should be stored at 4 °C upon arrival. With containers tightly closed, the product is stable for 12 months under recommended storage conditions. DO NOT FREEZE. Freezing may reduce the binding efficiency of the beads and result in lower yield.

1.3 Specifications

- CleaniBeads solution is delivered ready-to-use.
- Amount of starting DNA fragments (double stranded): 5 ng-1 µg
- To assure accurate pipetting, the sample volume should be ≥ 50 µl
- Size of DNA purification products: >100 bp
- Volume ratio: CleaniBeads selectively bind DNA fragments based on the volume ratio of beads and sample. Generally, an increase in volume ratio will favor binding of shorter DNA fragments to the beads. See Appendix for more information.

1.4 Safety instructions

Use good laboratory procedures and practices, always wear a suitable lab coat, disposable gloves, and protective goggles. No special precautions for handling are necessary.

1.5 Other materials required (not provided)

- 80% ethanol [freshly prepared]
- Molecular biology-grade water, TE or 10 mM Tris solution
- Magnetic rack for 1.5 ml tubes or 200 μl tubes

1.6 Product Use Limitations / Warranty

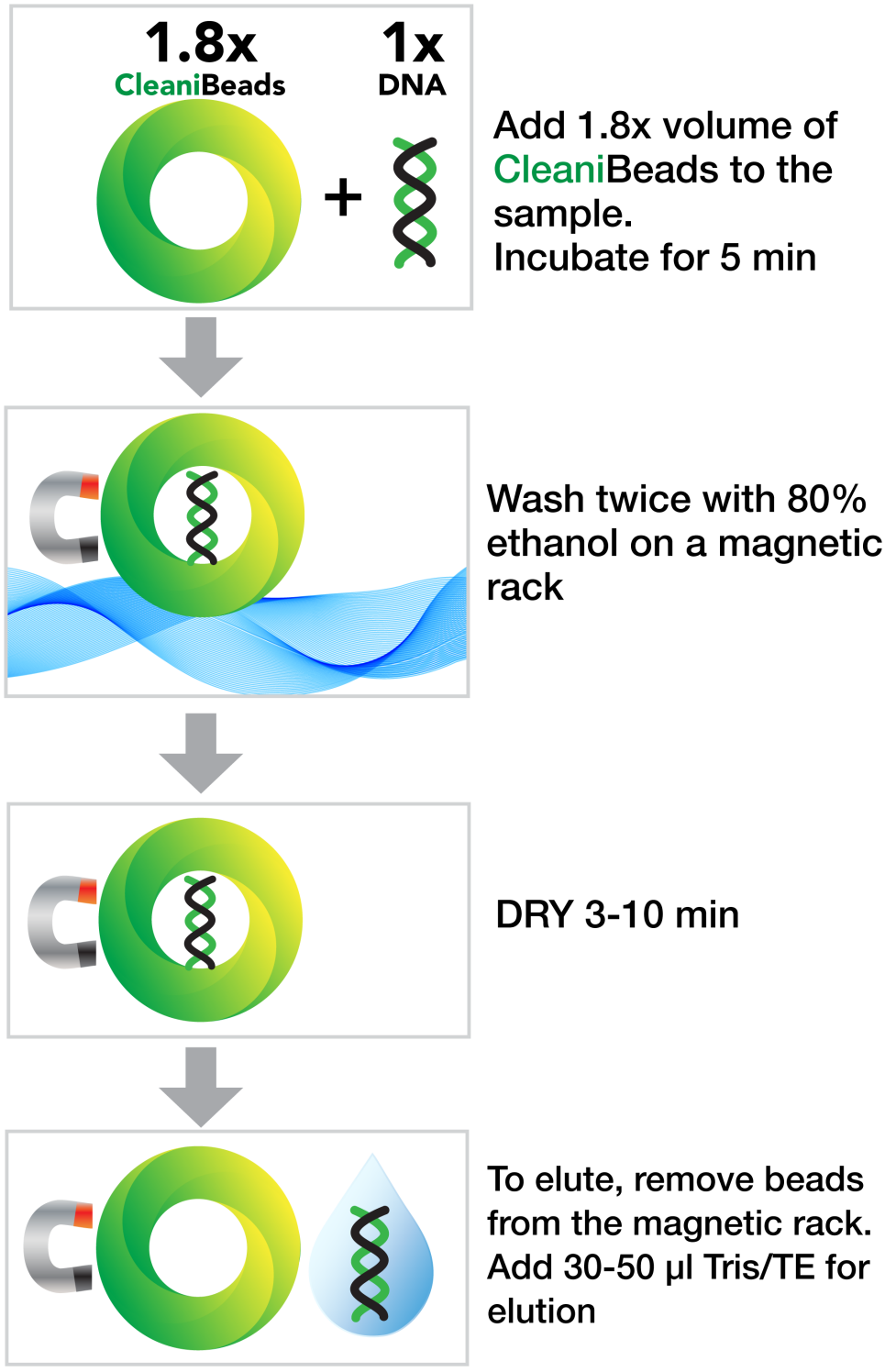
CleaniBeads are intended for Research Use Only. This product is not intended for diagnostic and any clinical use.

OncoSeek Ltd. shall only be responsible for the product specifications and the performance range of CleaniBeads according to the specifications of internal quality control, product documentation, and marketing materials. OncoSeek warrants to meet the stated specifications. Oncoseek's sole obligation and the customer´s sole remedy is limited to replacement of products in the event products fail to perform as warranted.

OncoSeek shall not be liable for claims for any other damages or strict liability arising in connection with the sale or the failure of CleaniBeads to perform in accordance with the stated specifications.

Product claims are subject to change. Please contact our technical support team for the most up-to-date information on CleaniBeads.

2. Simplified workflow for experienced users



3. Single-sided Size Selection Protocol (For purifying DNA >100 bp)

[Before starting] **Equilibrate beads to room temperature for 30 minutes before use**

1) Tighten the cap of CleaniBeads bottle. Vortex CleaniBeads until it is completely resuspended and appears homogenous.
2) To bind DNA to CleaniBeads, add 1.8X volume of CleaniBeads (e.g. 90 μl) to the sample (e.g. 50 μl). Immediately mix them thoroughly by pipetting (10 times) or vortexing (30 seconds), and briefly spin down the mixture without pelleting the beads.
3) Incubate at room temperature for 5 minutes.
4) Pellet CleaniBeads on a magnetic rack until the supernatant is clear.
5) Carefully discard the supernatant without disturbing the beads.
6) Wash CleaniBeads by adding 200 μl 80% ethanol and incubate for 30 seconds. Carefully discard the supernatant without disturbing the beads.
7) Wash once more by repeating the step 6.
8) Open the lid to dry the beads for 3-10 minutes. (Do not over dry the beads. Over drying of the beads may reduce the yield.)
9) To elute, remove beads from rack and then resuspend beads in 30-50 μl molecular biology-grade water, TE or 10 mM Tris solution. Resuspend by carefully pipetting up and down for 10 times and incubate for 2 minutes at room temperature.
10) Pellet CleaniBeads on the magnetic rack for 3 minutes.
11) Carefully transfer the eluate to a new tube and store the eluate at -20°C prior to subsequent experiments.

4. Double-sided Size Selection Protocol

(For purifying 250-300 bp DNA fragments)

- This protocol is based on 50 µl DNA input
- The volume ratio of beads is calculated based on the original volume of DNA input

First Round of Bead Selection

- This round of bead selction is to remove unwanted large DNA (>300 bp)
- Unwanted large DNA fragments will bind to the beads. The DNA-beads complexes will be discarded in this step. The desired DNA fragments will be in supernatant

[Before starting] **Equilibrate beads to room temperature for 30 minutes before use**

1) Tighten the cap of CleaniBeads bottle. Vortex CleaniBeads until it is completely resuspended and appears homogenous.
2) Add 45 µl (0.9X) CleaniBeads to 50 µl DNA sample, mix thoroughly by pipetting (10 times) or vortexing (30 seconds), and briefly spin down the mixture without pelleting the beads.
3) Incubate at room temperature for 5 minutes.
4) Place the sample tube on a magnetic rack until the supernatant is clear.
5) Carefully transfer the supernatant to a new tube without disturbing the beads. **(Caution: Do not discard the supernatant; proceed to the 2nd round of bead selection with it)**

Second Round of Bead Selection

- This round of bead selection is to recover 250-300 bp DNA fragments from the supernatant collected at the step 5.

6) Add 10 μl (0.2X) resuspended CleaniBeads to the collected supernatant.

7) Mix the mixture thoroughly and Incubate at room temperature for 5 minutes.

8) Pellet CleaniBeads on a magnetic rack until the supernatant is clear.

9) Carefully discard the supernatant without disturbing the beads.

10) Keep tube on the magnetic rack. Wash CleaniBeads by adding 200 μl 80% ethanol and incubate for 30 seconds. Carefully discard the supernatant without disturbing the beads.

11) Wash once more by repeating the step 10.

12) Open the lid to dry the beads for 3-5 minutes. (Do not over dry the beads. Over drying of the beads may reduce the yield.)

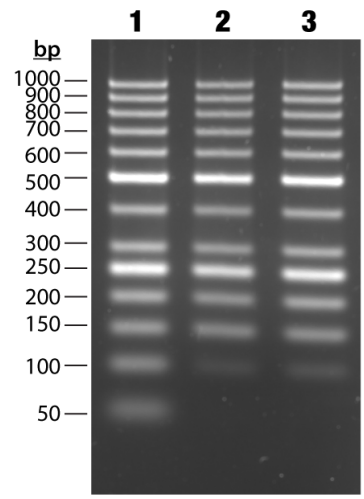
13) To elute, remove the sample from rack and then resuspend beads in 30-50 μl molecular biology-grade water, TE or 10 mM Tris solution. Resuspend by carefully pipetting up and down for 10 times and incubate for 2 minutes at room temperature.

14) Pellet CleaniBeads on the magnetic rack for 3 minutes.

15) Carefully transfer the eluate to a new tube and store the eluate at -20°C prior to subsequent experiments.

5. Quality control

Visualization of the pre- and post-purified samples on agarose gel stained with ethidium bromide or SYBRSafe DNA stain is recommended for a semi-quantitative estimation of recovery. Please see an example below:

CleaniBeads, Lot: 8021001 Lane 1: DNA ladder (pre-purified) Lane 2: DNA ladder (post-purified using CleaniBeads) Lane 3: DNA ladder (post-purified using magnetic beads from another supplier)	

6. Frequently asked questions

Questions	Answers
1. The colors of DNA-CleaniBeads mixture are not consistent across multiple samples.	1. **Completely vortex to mix CleaniBeads right before use.**
2. My downstream reactions or procedures do not work properly after performing purification.	2. **The pellet should be dried completely. Residual ethanol in the eluate will inhibit or hinder your downstream applications.**
3. How to determine if the beads are dried?	3. **The beads look shiny when they are wet and will turn dull when they get dried.**
4. How do I know the beads are overdried?	4. **The beads pellet will crack when they are overdried.**
5. Is there any size limitation to the DNA that can bind with CleaniBeads?	5. **DNA larger than 100 bp can be recovered. Please refer the attached quality control gel image.**
6. The purification has low yield.	6. **Ethanol wash solution should be freshly prepared.**
7. What kind of magnet should I use for automation on 96-well plate?	7. **It depends on your reaction scale and plate. If working on deep well plate, one may consider magnetic head like Pharma KingFisher™ Flex Magnetic Head (Applied Biosystems, A31542). If working on 0.2ml PCR plates, MAGNUM™ EX Universal Magnet Plate (Alpaqua Engineering) can be considered. We recommend using magnets that will concentrate beads against the wall rather than those concentrate beads at the exact bottom of the plate.**
8. How do I dry CleaniBeads in automation process?	8. **After aspiring ethanol solution, incubate the plate at 37-55°C for a few minutes to dry the beads. Avoid over drying.**
9. What volume of washing solution should I use for automation?	9. **Avoid reaching the volume capacity of your plate to prevent spilling and cross contamination of samples.**

7. Ordering information

Product	Volume (ml)	Catalog no.
CleaniBeads XS	2	CB-2ML
CleaniBeads S	10	CB-10ML
CleaniBeads M	25	CB-25ML
CleaniBeads L	50	CB-50ML

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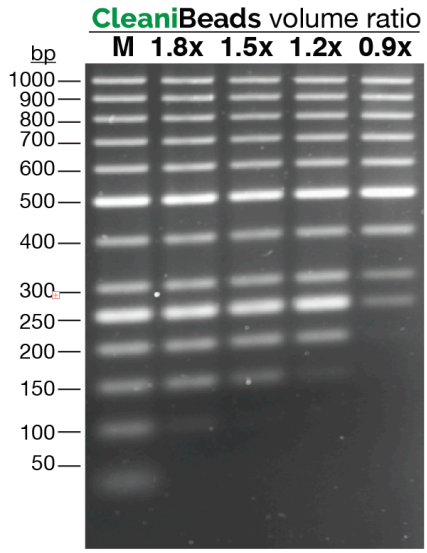
8. Appendix

(A) Adjusting the ratio of CleaniBeads and DNA samples allows specific size selection of DNA fragments, as demonstrated below.

CleaniBeads, Lot: 8021001 Lane 1: DNA ladder (pre-purified) Lane 2: CleaniBeads purification with 0.9x vol ratio Lane 3: CleaniBeads purification with 1.2x vol ratio Lane 4: CleaniBeads purification with 1.5x vol ratio Lane 5: CleaniBeads purification with 1.8x vol ratio	

(B) Double-sided size selection of DNA fragments

CleaniBeads, Lot: 8021001 Lane 1: DNA ladder (pre-purified) Lane 2: 250-300 bp dsDNA fragments purification with CleaniBeads after two rounds of bead selection (1st round volume ratio: 0.9X; 2nd round: 0.2X)