
DNBSEQ DNA Clean Beads User Manual

Cat. No.: 940-001281-00 , 940-001282-00
940-001283-00 , 940-001284-00

About the user manual

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Revision history

Manual Rev	Kit version	Date	Description
1.0	V1.0	Jul. 2023	Initial release

 Please use the latest version of the manual and use it with the corresponding kit.

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1 Product overview

1.1 Introduction

DNBSEQ DNA Clean Beads are used for purification and size selection of DNA samples. Our optimized buffer system recovers DNA products above 100 bp. DNBSEQ DNA Clean Beads are compatible with DNA and RNA library construction kits of various brands. They are used in exactly the same way as AMPure XP Beads. The purification yield and fragment size distribution are consistent with those of AMPure XP Beads. DNBSEQ DNA Clean Beads can be used in place of AMPure XP Beads.

1.2 Intended use

Intended for various brands of DNA and RNA next-generation sequencing library construction kits.

1.3 Components

This kit comes in 4 specifications: 3.2 mL, 8 mL, 15 mL, and 50 mL. For component details, refer to the following table.

Table 1 DNBSEQ DNA Clean Beads (3.2 mL) (Cat. No.: 940-001284-00)



Item & Cat. No.	Component	Cap color	Spec & Quantity
DNBSEQ DNA Clean Beads Cat. No.: 940-001284-00	DNA Clean Beads	 White	3.2 mL/tube × 1
	TE Buffer	 White	3.2 mL/tube × 1

Table 2 DNBSEQ DNA Clean Beads (8 mL) (Cat. No.: 940-001282-00)



Item & Cat. No.	Component	Cap color	Spec & Quantity
DNBSEQ DNA Clean Beads Cat. No.: 940-001282-00	DNA Clean Beads	 White	8 mL/tube × 1
	TE Buffer	 White	4 mL/tube × 1

Table 3 DNBSEQ DNA Clean Beads (15 mL) (Cat. No.: 940-001283-00)






Item & Cat. No.	Component	Cap color	Spec & Quantity
DNBSEQ DNA Clean Beads Cat. No.: 940-001283-00	DNA Clean Beads	 White	15 mL/tube × 1
	TE Buffer	 White	17 mL/tube × 1

Table 4 DNBSEQ DNA Clean Beads (50 mL) (Cat. No.: 940-001281-00)

Item & Cat. No.	Component	Cap color	Spec & Quantity
DNBSEQ DNA Clean Beads Cat. No.: 940-001281-00	DNA Clean Beads	 White	50 mL/tube × 1
	TE Buffer	 White	25 mL/tube × 1

1.4 Storage and transportation

DNBSEQ DNA Clean Beads

- Storage temperature: 2 °C to 8 °C. **Do not freeze.**
- Transportation temperature: 2 °C to 8 °C
 -  Production date and expiration date: refer to the label.
 - With proper transport, storage, and use, all components can maintain complete activity within their shelf life.

1.5 User-supplied materials

Table 5 User-supplied equipment list

Equipment	Recommended brand
Vortex mixer	/
Desktop centrifuge	/
Pipettes	/
Magnetic rack for 1.5 mL tubes or 0.2 mL tubes or 96-well plate	/

Table 6 Recommended reagent/consumable list

Reagent/consumable	Recommended brand
Nuclease Free (NF) water	/
100% Ethanol (Analytical Grade)	/
Pipette tips	/
1.5 mL tube	/
0.2 mL PCR tube or 96-well plate	/

1.6 Precautions and warnings

1.6.1 Before use

- This product is for research use only, not for in vitro diagnosis. Please read this manual carefully before use.
- Remove the magnetic beads from the 4 °C refrigerator 30 min in advance to allow the beads to equilibrate to room temperature. Equilibrating to room temperature ensures the beads are at the expected capture efficiency.
- Before each use, vortex or pipette the beads to ensure that they are thoroughly mixed.
- The amount of magnetic beads used is usually expressed by a multiplier, "x", which represents the ratio of the volume of magnetic beads to the original volume of the sample.

For example, the original volume of a sample is 50 µL.

If using a "1 × Beads" to cleanup, the volume of "1 × Beads" is $1 \times 50 \text{ µL} = 50 \text{ µL}$.

If using a "0.8 × 1st Beads + 0.2 × 2nd Beads" to select a specific DNA fragment size. For the 1st step, the volume of "0.8 × Beads" is $0.8 \times 50 \text{ µL} = 40 \text{ µL}$. For the 2nd step, the volume of "0.2 × Beads" is $0.2 \times 50 \text{ µL} = 10 \text{ µL}$.

- The volume of the beads used during cleanup determines the lower size limit of the fragment that can be purified. A higher volume of beads used allows for selection of a smaller fragment size.
For example, using "1 x Beads" allows for selection of target fragments larger than 200 bp, while using "2 x Beads" allows for selection of target fragments larger than 100 bp.
- It is recommended that you use pipette tips with filters to prevent cross-contamination. Use a new tip each time for pipetting different solutions or samples.
- Avoid skin and eyes contact with samples and reagents. Do not eat or drink the samples and reagents. In case of contact with skin and eyes, rinse immediately with plenty of water and seek medical advice.
- Conform to the law and regulations when disposing of all samples and reagents.
- If you have questions, please contact Technical Support.

1.6.2 Operation notes

- **Sample volume:** If the sample volume decreases (for example, from evaporation during incubation), add TE Buffer to reach the recommended sample volume. Purify the sample with the recommended volume of magnetic beads.
- **Uncapping:** Carefully open or close the tube cap while keeping the tube on the magnetic rack. Strong vibrations may cause sample loss from liquid or beads spilling out of the tubes. It is recommended that you hold the middle or lower part of the tube when opening the cap.

Removing the supernatant

1. Mix the sample and magnetic beads by vortexing or gently pipetting. Place the tube on a magnetic rack for separation. Do not remove the supernatant until the solution is completely clear.
2. The separation process takes approximately 2 to 5 min. Considering the difference in magnetism of magnetic racks or plates, leave enough time for the solution to become completely clear.
3. Keep the centrifuge tube(s) on the magnetic rack when removing the supernatant. Place the tip on the tube wall that is away from the rack and bead pellet.
4. Leave 2 to 3 μ L liquid in the tube to avoid touching or removing the magnetic beads. Pipette all of the solution and beads back into the tube and restart the separation process if necessary.

Ethanol wash

1. Wash the beads with freshly prepared room temperature 80% ethanol. Sufficient ethanol should be added to immerse the beads entirely.
2. Keep the centrifuge tube(s) on the magnetic rack during washing. Do not shake or disturb the beads while washing.
3. Wash the beads twice and remove all remaining ethanol with a low-volume pipette. If liquid remains on the tube wall, centrifuge the tube briefly and separate the beads from the liquid on the magnetic rack. Remove all remaining liquid with a low-volume pipette.

Air-dry

1. After both ethanol wash steps, air-dry the beads at room temperature.
 - The surface of the magnetic beads is **cracking**: Indicates over-drying and reduces the purification yield.
 - The surface of the magnetic beads is reflective: Indicates insufficient drying and easily causes anhydrous ethanol residues and affects subsequent reactions.
 - The surface of the magnetic beads is matte: Indicates that the beads are completely dry.
2. Air-drying takes approximately 3 to 5 min at room temperature. Different indoor temperatures and humidities of the air in labs may affect the drying time. Watch closely until the beads appear to have a matte appearance, which indicates complete drying.

Elution

1. Elute with the TE Buffer included with the kit.
2. The elution volume of TE buffer should be 2 μ L more than the pipetting volume of the supernatant to avoid touching or pipetting the magnetic beads.

1.7 Workflow

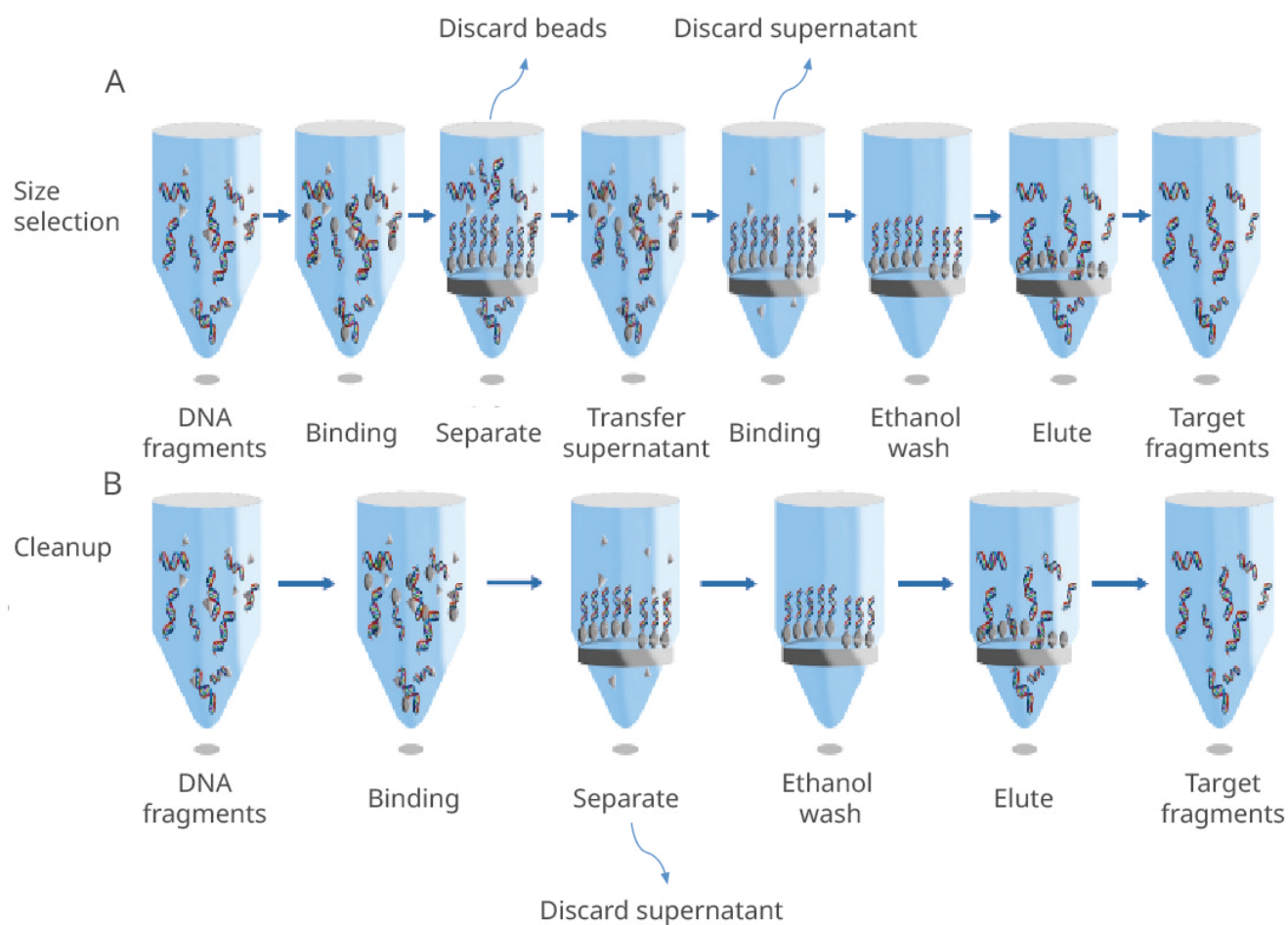







Figure 1 Size selection and cleanup process overview

Section	Workflow	Total time	Hands-on time
A	Size selection 	40 - 50 min	25 - 35 min
B	Cleanup 	30 - 40 min	20 - 30 min

-  Total time: The theoretical use time of 8 reactions. The time will be extended if the number of reactions increases.
- Hands-on time: The total required hands-on time in the process.
-  : The stop point.

2 Protocol

2.1 Size selection

-  Before operation, carefully read “Size selection conditions” on page 10.
For example, using a (0.8 ×) 1st Beads + a (0.2 ×) 2nd Beads with DNA Clean Beads for a 50 µL DNA fragments will result in a target fragment peak size of 280 bp.
- If using a different set of recommended size selection steps, or if using different volume of DNA fragments other than 50 µL, adjust the volume of DNA Clean Beads used accordingly.

2.1.1 Preparation

Table 7 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied; freshly prepared.
TE Buffer	Place at room temperature (RT).
DNA clean beads	Take out 30 min in advance to equilibrate to RT. Mix thoroughly by vortexing before each use.

2.1.2 Size selection

- Transfer **50 µL of DNA fragment** to a new 1.5 mL centrifuge tube. Add **TE Buffer** for a total volume of 50 µL if the volume of DNA fragments sample is not enough.
- Mix the DNA Clean Beads thoroughly. Add **40 µL (0.8 ×) of DNA Clean Beads** to each sample tube. Gently pipette at least 10 times until all beads are suspended. Ensure that all of the solution and beads in the tip are transferred into the tube after mixing. Or, mix with a vortexer.

3. Incubate the sample(s) at room temperature for 5 min.

i In the next step, keep the supernatant and discard the beads.

4. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer 50 μ L supernatant to a new 1.5 mL centrifuge tube.

5. Add **10 μ L (0.2 \times) of DNA Clean Beads** to each sample tube (contain 50 μ L of supernatant). Gently pipette at least 10 times until all beads are suspended. Or, mix with a vortexer.

6. Incubate the sample(s) at room temperature for 5 min.

7. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully remove and discard the supernatant.

8. While keeping the tube(s) on the magnetic rack, add **200 μ L of 80% ethanol** to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.

9. Repeat step 8. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.

10. Keep the tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.

i Over-drying the beads will result in reduced yield.

11. Remove the tube(s) from the magnetic rack and add **42 μ L of TE Buffer** to elute the DNA. Gently pipette at least 10 times until all beads are suspended. Or, mix with a vortexer.

12. Incubate the sample(s) at room temperature for 5 min.

13. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer **40 μ L** of supernatant to a new 0.2 mL PCR tube.

|| Stop point Products can be stored at -20 $^{\circ}$ C.

2.2 Cleanup



i For 50 μ L of DNA fragments, the target fragment peak size will be above 150 bp when purifying with 1 \times DNA Clean Beads.

2.2.1 Preparation

Table 8 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied; freshly prepared.
TE Buffer	Place at RT.
DNA Clean Beads	Take out 30 min in advance to equilibrate to RT. Mix thoroughly by vortexing before each use.

2.2.2 Cleanup

1. Transfer **50 µL of DNA fragments** to a new 1.5 mL centrifuge tube. Add **TE Buffer** for a total volume of 50 µL if the volume of DNA fragments sample is not enough.
2. Mix the DNA Clean Beads thoroughly. Add **50 µL (1 ×) of DNA Clean Beads** to each sample tube. Gently pipette at least 10 times until all beads are suspended. Ensure that all of the solution and beads in the tip are transferred into the tube after mixing. Or, mix with a vortexer.
3. Incubate the sample(s) at room temperature for 5 min.
4. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully remove and discard the supernatant.
5. While keeping the tube(s) on the magnetic rack, gently add **200 µL of 80% ethanol** to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.
6. Repeat step 5. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
7. Keep the tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.
 Over-drying the beads will result in reduced yield.
8. Remove the tube(s) from the magnetic rack and add **42 µL of TE Buffer** to elute the DNA. Gently pipette at least 10 times until all beads are suspended. Or, mix with a vortexer.
9. Incubate the sample(s) at room temperature for 5 min.
10. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully transfer **40 µL** of supernatant to a new 0.2 mL PCR tube.
 **Stop point** After cleanup, the product(s) can be stored at -20 °C.

Appendix 1 Size selection conditions

Select PCR products whose fragments are distributed between 100 bp to 1500 bp.

The following table shows the different target fragment peak size with different size selection conditions.

Table 9 Recommended size selection conditions

Target fragment peak size (bp)	180	230	280	335	420	550
1 st Beads (×)	1	0.9	0.8	0.7	0.6	0.5
2 nd Beads (×)	0.5	0.2	0.2	0.2	0.2	0.2

The different size selection conditions of the Agilent 2100 bioanalyzer results are presented in the following figure.

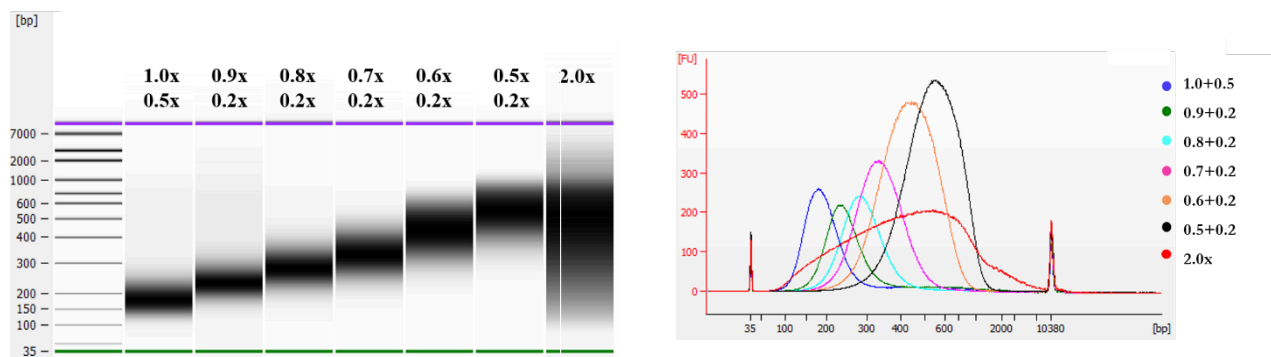


Figure 2 Results of different size selection conditions

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