ZYMO RESEARCH

DNA Purification Made Simple

Select-a-Size DNA Clean & Concentrator™ MagBead Kit

Purify desired range of DNA fragment sizes from library preps, PCR, endonuclease digestions, ligations, etc.

Highlights

- **Tunable**: High quality tunable DNA fragment size selection 150–900 bps
- **Consistent Performance:** Rapid purification with high reproducibility
- **Automation Ready**: Automation friendly and scalable protocol

Catalog Numbers:
D4084, D4085

Scan with your smart-phone camera to view the online protocol/video.

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Product Contents

Select-a-Size DNA Clean & Concentrator ™ MagBead Kit	D4084 (10 ml)	D4085 (50 ml)	Storage Temperature
Select-a-Size MagBeads	10 ml	50 ml	4-8 °C
DNA Wash Buffer [1]	24 ml	3 x 24 ml	Room Temp.
DNA Elution Buffer	16 ml	50 ml	Room Temp.
Instruction Manual	1	1	-

[1] Ethanol must be added prior to use as indicated on **DNA Wash Buffer** label.

Specifications

- **DNA Purity –** Eluted DNA is of high quality and is well suited for ligations, restriction digestions, library preparation cleanup, and next generation sequencing applications.

- **Recovery Volume –** ≥ 25 µl of **DNA Elution Buffer** for 96 well plates. ≥ 10 µl of **DNA Elution Buffer** for manual preps performed using microcentrifuge tubes.

- **Required Equipment –** Magnetic Separator.

- **Processing Time –** ≥ 10 mins.

- **Materials Needed, but not Provided –** clean microcentrifuge tube or 96-well plates for elution.

Principle of Technology

The **Select-a-Size DNA Clean & Concentrator™ MagBead Kit** works on the principle of selective binding, wherein the size of the nucleic acid and the ratio of the magnetic beads controls what is retained on the beads and what remains in the supernatant. Either fraction (beads or supernatant) can be further purified which is an enabling and flexible feature of this technology. As the ratio of MagBeads to sample increases, proportionally lower molecular weight DNA (smaller fragments) are retained. Therefore, the size selection is controlled by increasing or decreasing quantities of MagBeads. Samples can be size selected to remove smaller fragments with Left-Sided Size Selection, larger fragments with Right-Sided Size Selection (**Figure 1**), or both large and small fragments in Double-Sided Size Selection. Listed within this protocol are the most common cutoffs and starting sample volumes. **Cutoffs not included within this protocol can be determined by titrating between points**.

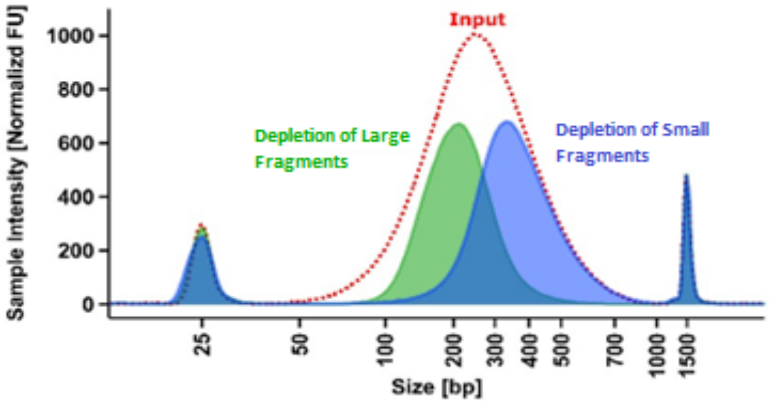

Figure 1. Depletion of large fragments (green) and depletion of small fragments (blue) on sonicated salmon sperm DNA (red) DNA size was selected according to the Select-a-Size DNA Clean & Concentrator™ MagBeads protocol and the results were analyzed by Agilent 4150 TapeStation. 50 µl of sonicated salmon sperm DNA at a concentration of 8.0 ng / µl in water was used as a standard input to evaluate size selection efficiency and cutoff.

Product Description

The **Select-a-Size DNA Clean & Concentrator™ MagBead Kit** provides the fastest and easiest method to purify specific ranges of DNA fragments from PCR, endonuclease digestions, ligations, library preparations, adapter removal, etc. This simple workflow allows for specific cutoffs that can be modified to suit reaction clean-up, left-sided, right-sided, or even double-sided size selection. The **Select-a-Size MagBeads** selectively binds fragments based on the volume added relative to the sample. Simply choose a desired cutoff to bind the target of interest onto the beads and remove species outside of this range. (**Figure 2**) The desired DNA is easily eluted from the beads following a rapid wash regimen.

Choose from one of the pre-determined cutoffs or fine-tune the protocol for even more specific selections between these points. Size selections can be performed in as little as 10 minutes to yield high-quality DNA which is suitable for highly sensitive applications including Next Generation Sequencing, and any other sensitive downstream applications. The protocol can be performed manually or using an automated platform for high throughput processing.

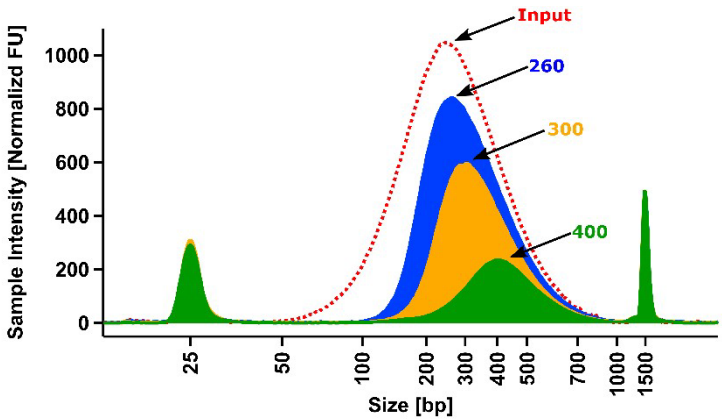

Figure 2. Select-a-Size DNA Clean & Concentrator™ MagBead Kit demonstrating depletion of small fragments at 260 bp (blue trace), 300 bp (yellow trace), 400 bp (green trace). DNA size was selected according to the Select-a-Size DNA Clean & Concentrator™ MagBead kit protocol and the results were analyzed by Agilent 4150 TapeStation. 50 µl of sonicated salmon sperm DNA at a concentration of 9.0 ng/µl in water was used as a standard input to evaluate size selection efficiency and cutoff.

Protocol

Buffer Preparation

Before starting: Add 96 ml of 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA Wash Buffer** concentrate.

Clean up and Depletion of Smaller Fragments (Left-Sided Selection):

The following procedure should be performed at room temperature (20–30°C).

1. Resuspend the magnetic particles by vigorously shaking the **Selecta-Size MagBeads** until homogenous.[2]

2. Choose the desired peak from the **Table 1** below.[3] For a sample volume of 50 µL, determine the amount of **Select-a-Size MagBeads** required.

   Note: (Sample Volume) x (Ratio) = Volume of Select-a-Size MagBeads

Peak DNA Fragments Retained	Ratio of Select-a-Size MagBeads	Volume of MagBeads for 50 µl starting sample	Remaining Fragments after Selection
260	1.24	62	>100bp
300	0.98	49	>150bp
400	0.76	38	>200bp

   Table 1. This table shows the recommended titration volumes for a 50 µL sample to retain the peak DNA fragments listed. Additional ratio selections can be found in the Appendix (pg. 10-11).

3. Add the necessary volume of **Select-a-Size MagBeads** to the sample. Mix thoroughly by pipetting or vortexing until homogenous. Incubate for 5 minutes at up to 30 °C.[4]

4. Place the sample on a magnetic rack or plate and incubate for 5 minutes, or until the magnetic beads have fully separated from solution.

[2] For complete resuspension, allow **Select-a-Size MagBeads** to equilibrate to room temperature (20-30°C).

[3] For complete adapter/dimer removal, select a cutoff that is at least 50 bp above the undesired fragment size.

[4] For maximum recovery and consistent yields, incubation at 30 °C is recommended but not required at any step.

5. Once the beads have been cleared from solution, remove, and discard the supernatant.[5]

6. While the beads are still on the magnetic rack, add 200 µl of **DNA Wash Buffer**. Remove and discard the supernatant. Repeat this step.

7. While the beads are still on the magnetic rack, aspirate any residual **DNA Wash Buffer**. Remove samples from the magnetic rack.

   Note: An optional drying incubation of 3 minutes at room temperature can be performed to ensure all traces of ethanol are removed.

8. Add ≥ 25 µl **DNA Elution Buffer** to the beads[6,7] and mix thoroughly by pipetting up and down or vortexing until homogenous. Incubate at up to 30°C for 2 minutes.

9. Place the sample on a magnetic rack for 1–2 minutes to separate the magnetic beads from eluate.

10. Transfer supernatant to a clean microcentrifuge tube or 96-well plate. The ultrapure DNA is now ready for use.

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[5] Avoid aspirating any beads when removing the supernatant. To best prevent this, leave 2-5 µl of liquid in the sample container.

[6] For plates, an elution volume greater than 25 µl may be required to guarantee full contact with the magnetic beads.

[7] For microcentrifuge tubes, an elution volume as low as 10 µl can be used to resuspend the beads and obtain a highly concentrated eluate.

Depletion of Larger Fragments (Right-Sided Selection):

Important! *See* ***Buffer Preparation*** *on page* ***5*** *before starting.*
The following procedure should be performed at room temperature (20–30 °C).

1. Resuspend the magnetic particles by vigorously shaking the **Select-a-Size MagBeads** until homogenous.[8]

2. Bring the DNA sample volume up to 50 µl with **DNA Elution Buffer**.

3. Choose the desired cutoff from **Table 2** below. Add the necessary **first volume** of **Select-a-Size MagBeads** to the sample based on the desired ratio. Mix thoroughly by pipetting or vortexing until homogenous. Incubate this mixture for 5 minutes at up to 30 °C.
   Note: (Sample Volume) x (Ratio) = Volume of Select-a-Size MagBeads

DNA Fragments Depleted	First ratio of Select-a-Size MagBeads	First Volume of MagBeads (µl)	Second Ratio of Select-a-Size MagBeads	Second Volume of MagBeads (µl)
>200	0.96	48	0.84	42
>300	0.8	40	1	50
>400	0.72	36	1.08	54
>600	0.6	30	1.2	60
>700	0.56	28	1.24	62
>900	0.52	26	1.28	64

Table 2. This table shows the recommended titration volumes for a 50 µL sample to deplete DNA fragments above the size listed. Volume titration for further cutoffs can be calculated by changing ratio one (R1) to desired cut off and adjusting ratio 2 (R2) so that R1+R2=1.8.

4. Place the sample on a magnetic rack or plate and incubate for 5 minutes, or until the magnetic beads have fully separated from solution.

5. Once the beads have been cleared from solution, **transfer the supernatant into a new tube**.[9] Discard the beads.

6. Refer to **Table 2** and add the **second volume** of **Select-a-Size MagBeads**, corresponding to cutoff chosen on step 3, to the supernatant from step 5. Mix thoroughly by pipetting or vortexing until homogenous. Incubate this mixture for 2 minutes at up to 30 °C.

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[8] For complete resuspension, allow **Select-a-Size MagBeads** to equilibrate to room temperature (20-30°C).
[9] Avoid aspirating any beads when removing the supernatant. To best prevent this, leave 2-5 µl of liquid behind.

Example: To retain fragments smaller than 300 bp with a 50 µl starting sample, 40.0ul of beads should be added on step 3 and then 50.0 µl added to the supernatant obtained from step 5.

7. Place the sample on a magnetic rack or plate and incubate for 5 minutes, or until the magnetic beads have separated from solution.

8. Once the beads have cleared from solution, discard the supernatant.[10]

9. While the beads are still on the magnetic rack, add 200 µl of **DNA Wash Buffer**. Remove and discard the supernatant. Repeat this step.

10. While the beads are still on the magnetic rack, aspirate any residual **DNA Wash Buffer**. Remove samples from the magnetic rack.

    Note: An optional drying incubation of 3 minutes at room temperature can be performed to ensure all trace of ethanol is removed.

11. Add ≥ 25 µl **DNA Elution Buffer** to the beads[11, 12] and mix thoroughly by pipetting up and down or vortexing until homogenous. Incubate at up to 30 °C for 2 minutes.

12. Place the sample on a magnetic rack and incubate for 2 minutes, or until the magnetic beads have separated from solution.

13. Transfer supernatant to the final tube or plate. The ultra-pure DNA is now ready for use.

[10] Avoid aspirating any beads when removing the supernatant. To best prevent this, leave 2-5 µl of liquid behind.

[11] For plates, an elution volume greater than 25 µl may be required to guarantee full contact with the magnetic beads.

[12] highly concentrated eluate. For microcentrifuge tubes, an elution volume as low as 10 µl can be used to resuspend the beads and obtain a

Double-Sided Size Selection:

Important! *See* ***Buffer Preparation*** *on page* ***5*** *before starting.*

The following procedure should be performed at room temperature (20–30°C).

To perform a double-sided selection, first perform a large fragment depletion and then perform a short fragment depletion on the same sample:

1. Choose the desired large fragments depletion ratio from **Table 2** above or as determined by titration.

2. Perform the large fragments depletion following the steps on page 7–8.
   Note: For step 11 (pg 8) elute with 50 µL **DNA Elution buffer.**

3. Choose the desired small fragment depletion ratio on **Table 1** on pg. 5 or as determined by titration (additional ratios shown in the appendix, pg 10-11, **Table 3**).

4. Perform a small fragment depletion on the eluate obtained on step 2 following the steps on page 5–6. Elute with ≥ 25 µl **DNA Elution buffer.**

5. Transfer supernatant to the final tube or plate. The ultra-pure DNA is now ready for use.

Appendix

Automation Scripts

The **Select-a-Size DNA Clean & Concentrator™ MagBead Kit** is compatible with automated platforms. For automation scripts and related technical support, email automation@zymoresearch.com. In the subject line, please include "Automation Scripts" and include the instrument used and the product catalog number.

Additional Cutoff Ratios for Short Fragment Depletion (Left-Sided Selection).

Volume of Magbeads (µl)	Ratios
30	0.60
32	0.64
34	0.68
36	0.72
38	0.76
40	0.8
42	0.84
44	0.88
46	0.92
48	0.96
52	1.04
56	1.12
58	1.16
62	1.24
64	1.28
72	1.44
74	1.48

Table 3. Various ratios were tested for short fragment Depletion with a 50 µl input of 6X sonicated salmon sperm DNA.

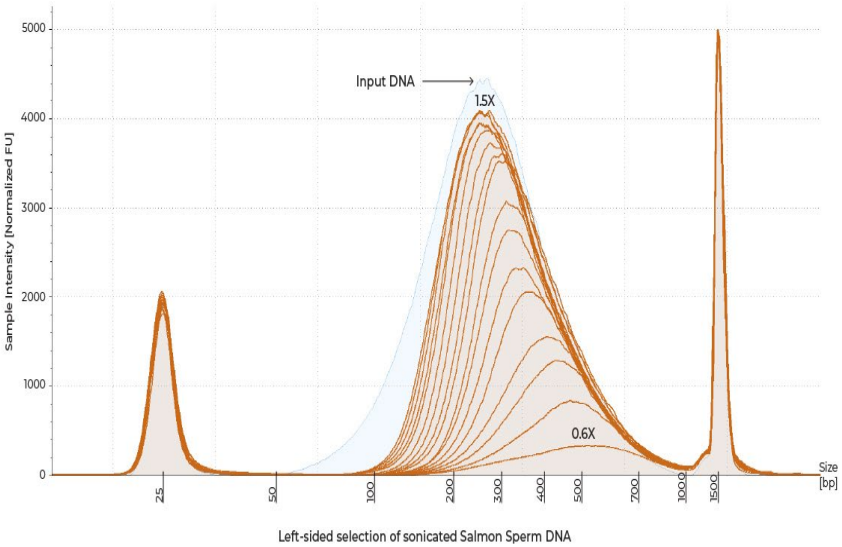

Figure 3. Additional Short Fragment Depletion (left sided selection) Ratios. The ratios shown on table 3 were analyzed with Agilent 4150 TapeStation. 6X sonicated salmon sperm DNA was used as input at an initial concentration of 75ng/µl.

Troubleshooting

Problem	Possible Causes and Suggested Solutions
Choosing Your Cutoff	
Titrating New Peaks or Cutoffs	• Alternative cutoffs can be identified by fine adjustments of the volume of **Select-a-Size MagBeads** added to the sample. The peaks and cutoffs provided within the protocol can be used as a guideline to pinpoint more specific cutoffs. In general, as the ratio of **Select-a-Size MagBeads** to sample increases, the peak or cutoff shifts to the left.
Inaccurate Size Selection	
Inconsistent Size Selection	• **Select-a-Size MagBeads not mixed thoroughly with sample**. Be sure to mix the MagBeads with the sample very well by pipetting or vortexing until homogenous. • **Stray Droplets**. Cutoffs can be very sensitive to slight changes in the ratio of **Select-a-Size MagBeads** to sample for cutoffs at higher molecular weights. Be vigilant of stray droplets being carried over into the sample. • **Volume of sample**. The sample volume should be at least 50 µl before adding **Select-a-Size MagBeads**. A lower volume is more prone to effects from pipetting inaccuracy which shifts the size selection.
Tailing of large size range in Right/Double-Sided Size Selection	• **Incomplete Separation**. Allow magnetic beads to completely separate from solution before aspirating out any liquid. The solution should be completely clear with a pellet of beads on the side of the t**ube**. Take care not to aspirate out any beads in the first supernatant removal. At this step, the larger fragments are bound onto the beads. To avoid removing these beads, leave 2-5 µl of buffer behind during the first supernatant removal.

Inefficient removal of undesired smaller fragments	• **Incomplete Washing**. The undesired small fragments may be present within the wells of the t**ube**/well. To remove these fragments, be sure to perform both wash steps with DNA Wash Buffer thoroughly. • **Pipette Calibration**. Higher cutoffs can be highly sensitive to minor errors in pipetting. Ensure that pipettes are properly calibrated before performing protocol.
Low DNA Recovery	
DNA Wash Buffer	• Ethanol was not added to the **DNA Wash Buffer**. • **Ensure that the bottle cap is screwed on tightly** after each use to prevent evaporation of the ethanol over time.
Magnetic Separation	• **Incomplete Separation**. Allow magnetic beads to completely separate from solution before aspirating out any liquid. The solution should be completely clear with a pellet of beads on the side of the t**ube**. Take care not to aspirate out any beads as the fragments of interest are bound on the beads.
Inefficient Elution	• **Elution volume does not cover the beads**. The minimum elution volume for plates will vary based on the magnetic rack used. The level of the eluate should completely cover the magnetic beads for complete elution. • **Elution volume not large enough**. Decreasing the elution volume can lead to a decrease in the recovery. The smaller the elution volume, the more difficult it will be to aspirate the eluate without carrying beads. • **Beads not resuspended**. When performing the elution with small volumes, it is critical to locate the pellet and resuspend the magnetic beads.
Drying Time	• **Do not over dry the MagBeads**. For complete removal of ethanol, resuspend samples with **DNA Elution Buffer** within three minutes of drying. Extended drying times reduce the elution efficiency of the beads.

Inefficient Binding	• **Mix sample with MagBeads well and incubate for at least 2–5 minutes**. To maximize the recovery of DNA, samples should be mixed thoroughly and can be incubated at 30 °C for 5 minutes. For thorough mixing, pipette the entire reaction volume up and down 10 times or vortex sample for 30 seconds. These additional steps are most helpful for sample volumes larger than 50 µl.
Cutoff too close to target	• **The cutoffs are not distinct**. For most efficient recovery, choose a cutoff as far removed from the desired fragments as possible. *E.g.,* If the desired fragments are around 500 bp and the undesired fragments are at 50 bp, choose the 100 bp cutoff for maximum retention of the desired fragment while completely depleting the undesired fragment. Choosing a higher cutoff results in diminished recovery of the desired fragments.
Range of selection too small for double sided size selection	• **Target region too small.** For a double-sided size selection, the recovery of a target region decreases significantly as the target region decreases. To maximize recovery of the target range, the two cutoffs should be as far apart as possible in order to increase the recovery of the target range.
Low DNA Quality	
Low 260/230	• **Salt Contamination**. Ensure that both wash steps are performed to thoroughly remove any residual salt contamination. Incomplete washing will result in low 260/230 ratios. • **Ethanol Contamination**. To prevent ethanol contamination, remove as much DNA Wash buffer as possible before drying beads. Samples can be given a quick spin to bring down remaining buffer before transferring to magnetic rack to aspirate any residual buffer. Alternatively, allow samples to air dry at room temperature for 3 minutes before resuspending with **DNA Elution Buffer**.

Ordering Information

Product Description	Catalog No.	Size
Select-a-Size DNA Clean & Concentrator™ MagBead Kit	D4084	10 ml
Select-a-Size DNA Clean & Concentrator™ MagBead Kit	D4085	50 ml

Individual Kit Components	Catalog No.	Amount
Select-a-Size MagBead	D4084-4-10 D4084-4-50	10 ml 50 ml
DNA Wash Buffer (concentrate)	D4003-2-24 D4003-2-48	24 ml 48 ml
DNA Elution Buffer	D3004-4-10 D3004-4-16 D3004-4-50	10 ml 16 ml 50 ml
Collection Plate	C2002	2 Plates
Elution Plate	C2003	2 Plates
96-Well Plate Cover Foil	C2007-2 C2007-4 C2007-8	2 Foils 4 Foils 8 Foils
ZR-96 MagStand	P1005	1 Stand

Notes

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