

## BeaverBeads™ DNA Select Isolation

### Product Introduction

BeaverBeads™ DNA Select Isolation kit based on superparamagnetic beads can select nucleic acid fragment speedily and simply. The recovered nucleic acid fragment can be used for Next Generation Sequencing. This product is suitable for manual operation and high-throughput automated pipetting workstations.

### Product Information

| Product Information               | 70407-5 | 70407-60 | 70407-450 | Remarks                        |
|-----------------------------------|---------|----------|-----------|--------------------------------|
| BeaverBeads™ DNA Select Isolation | 5 mL    | 60 mL    | 450 mL    | Stored at 2~8℃, avoid freezing |

### Preparation

- 80% Ethanol ( freshly -prepared )
- Water ( molecular biology grade ) or 10mM Tris-HCl , pH 8.0 or TE ( 10mM Tris-HCl,pH8.0 , 1mM EDTA ) for DNA elution
- Vortex oscillator
- Magnetic separator : can choose BEAVER magnetic separator , Product No. 60303

Note:Sample volume should be  $\geq 50 \mu\text{L}$ . A low volume will decrease pipetting accuracy and lower the accuracy of selection range.

### Operation process

#### 1. Left Side Size Selection

The majority of the sample's size distribution should be larger than the selection point. Generally speaking, increasing the ratio of BeaverBeads™ DNA Select beads volume to sample volume will increase the efficiency of binding smaller fragments.

1.1 DNA binding : Shake or vortex the BeaverBeads™ DNA Select Isolation bottle to resuspend the beads. Following the trend depicted in Fig.1(Left), add the required volume of BeaverBeads™ DNA Select Isolation for the desired ratio to the sample. Mix the total volume by pipetting 10 times and incubate at RT for 5 minutes.

TIP: volume of sample \* ratio =volume of BeaverBeads™ DNA Select Isolation

Example:  $50 \mu\text{L}$  sample \*0.6  $\times$  ratio =  $30 \mu\text{L}$  BeaverBeads™ DNA Select Isolation

1.2 Place the reaction vessel on a magnetic separator to magnetize the BeaverBeads™ DNA Select Isolation. Let sit at room temperature until the beads are completely settle to the magnet. Aspirate and discard the cleared supernatant. Do not disturb the BeaverBeads™ DNA Select Isolation.

1.3 Ethanol washes: With the reaction vessel still on the magnet, add  $200 \mu\text{L}$  freshly -prepared 80% ethanol and incubate at RT for 30 seconds. Aspirate and discard the ethanol supernatant. Repeat this step once.

TIP: the second wash step should try to fully remove the ethanol.

1.4 Dry beads: Leave the reaction vessel still on the magnetic separator for 2-5 minutes to air dry the BeaverBeads™ DNA Select Isolation.

TIP: Ensure all traces of Ethanol are removed but take care not to over dry the bead ring (bead ring appears cracked) as this will significantly decrease elution efficiency.

1.5 Elute DNA : Remove the reaction vessel from magnetic separator and add  $30\text{-}40 \mu\text{L}$  Water ( molecular biology grade ) or Tris-HCl or TE. Mix the total elution volume by pipetting 10 times to resuspend the beads and incubate at RT for 3-5minutes. Place the reaction vessel on a magnetic separator to magnetize the BeaverBeads™ DNA Select Isolation. Let sit at room temperature until the beads are completely settle to the magnet. Transfer the cleared supernatant containing size selected sample to an appropriate storage vessel.

TIP: The elution volume of this product can be as low as  $20 \mu\text{L}$ , but attention should be paid that the elution efficiency is related to the elution volume, the more the elution volume, the more the total amount of the nucleic acid, but the lower the concentration.

#### 2. Right Side Size Selection

The majority of the sample's size distribution should be smaller than the selection point. As a general rule, increasing the ratio of BeaverBeads™ DNA Select beads volume to sample volume will decrease the efficiency of binding larger fragments.

2.1 First DNA binding: Shake or vortex the BeaverBeads™ DNA Select Isolation bottle to resuspend the beads. Following the trend depicted in Fig.1(Right), add the required volume of BeaverBeads™ DNA Select Isolation for the desired ratio to the sample. Mix the total volume by pipetting 10 times and incubate at RT for 5 minutes.

TIP: volume of sample \* ratio =volume of BeaverBeads™ DNA Select Isolation

Example:  $50 \mu\text{L}$  sample \*0.61  $\times$  ratio =  $30.5 \mu\text{L}$  BeaverBeads™ DNA Select Isolation

2.2 Place the reaction vessel on a magnetic separator to magnetize the BeaverBeads™ DNA Select Isolation. Let sit at room temperature until the beads are completely settle to the magnet. Transfer the clear supernatant, which contains the Right Side Size Selected sample, to a new reaction vessel. The reaction vessel with the remaining beads can be discarded.

2.3 Second DNA binding: Add the required volume of BeaverBeads™ DNA Select Isolation, using the calculation below ,to the supernatant from Step 2.2. This will bind the fragments in the supernatant to the new BeaverBeads™ DNA Select Isolation beads. Mix the total volume by pipetting 10 times and incubate at RT for 15 minutes.

TIP: volume of sample \*( 1.8 - ratio ) =volume of BeaverBeads™ DNA Select Isolation

Example:  $50 \mu\text{L}$  sample \*(1.8-0.61) =  $59.5 \mu\text{L}$  BeaverBeads™ DNA Select Isolation

2.4 Place the reaction vessel on a magnetic separator to magnetize the BeaverBeads™ DNA Select Isolation. Let sit at room temperature until the beads are completely settle to the magnet. Aspirate and discard the cleared supernatant. Do not disturb the BeaverBeads™ DNA Select Isolation.

2.5 Ethanol washes : With the reaction vessel still on the magnet, add 200  $\mu$  L freshly -prepared 80% ethanol and incubate at RT for 30 seconds. Aspirate and discard the ethanol supernatant. Repeat this step once.

**TIP:** the second wash step should try to fully remove the ethanol.

2.6 Dry beads: Leave the reaction vessel still on the magnetic separator for 2-5 minutes to air dry the BeaverBeads™ DNA Select Isolation.

**TIP:** Ensure all traces of Ethanol are removed but take care not to over dry the bead ring (bead ring appears cracked) as this will significantly decrease elution efficiency.

2.7 Elute DNA : Remove the reaction vessel from magnetic separator and add 30-40  $\mu$  L Water ( molecular biology grade ) or Tris-HCl or TE. Mix the total elution volume by pipetting 10 times to resuspend the beads and incubate at RT for 3-5minutes. Place the reaction vessel on a magnetic separator to magnetize the BeaverBeads™ DNA Select Isolation. Let sit at room temperature until the beads are completely settle to the magnet. Transfer the cleared supernatant containing size selected sample to an appropriate storage vessel.

**TIP:** The elution volume of this product can be as low as 20  $\mu$  L, but attention should be paid that the elution efficiency is related to the elution volume, the more the elution volume, the more the total amount of the nucleic acid, but the lower the concentration.

### 3. Double-ended size selection

As a general rule, the Left Side Size Selection ratio always needs to be greater than the Right Side Size Selection ratio. Instead of adding beads to achieve the full 1.8 $\times$  bead ratio(witch will recover ALL fragments>100bp), add just enough beads to achieve a final ratio for your desired lower end cutoff.

3.1 First DNA binding: Shake or vortex the BeaverBeads™ DNA Select Isolation bottle to resuspend the beads. Following the trend depicted in Fig.1(right), add the required volume of BeaverBeads™ DNA Select Isolation for the desired ratio to the sample. Mix the total volume by pipetting 10 times and incubate at RT for 5 minutes.

**TIP:** volume of sample \* Right side size selection ratio =volume of BeaverBeads™ DNA Select Isolation

Example: 50  $\mu$  L sample \*0.61 $\times$  ratio = 30.5  $\mu$  L BeaverBeads™ DNA Select Isolation

3.2 Place the reaction vessel on a magnetic separator to magnetize the BeaverBeads™ DNA Select Isolation. Let sit at room temperature until the beads are completely settle to the magnet. Transfer the clear supernatant, which contains the Right Side Size Selected sample, to a new reaction vessel. The reaction vessel with the remaining beads can be discarded.

3.3 Second DNA binding: Following the trend depicted in Fig.1(left),using the calculation below ,add the required volume of BeaverBeads™ DNA Select Isolation to the supernatant from Step 3.2. Mix the total volume by pipetting 10 times and incubate at RT for 15 minutes.

**TIP:** volume of sample \*( Left side size selection ratio - Right side size selection ratio ) =volume of BeaverBeads™ DNA Select Isolation

Example: 50  $\mu$  L sample \*(0.8-0.61) = 9.5  $\mu$  L BeaverBeads™ DNA Select Isolation

3.4 Place the reaction vessel on a magnetic separator to magnetize the BeaverBeads™ DNA Select Isolation. Let sit at room temperature until the beads are completely settle to the magnet. Aspirate and discard the cleared

supernatant. Do not disturb the BeaverBeads™ DNA Select Isolation.

3.5 Ethanol washes : With the reaction vessel still on the magnet, add 200  $\mu$  L freshly -prepared 80% ethanol and incubate at RT for 30 seconds. Aspirate and discard the ethanol supernatant. Repeat this step once.

**TIP:** the second wash step should try to fully remove the ethanol.

3.6 Dry beads: Leave the reaction vessel still on the magnetic separator for 2-5 minutes to air dry the BeaverBeads™ DNA Select Isolation.

**TIP:** Ensure all traces of Ethanol are removed but take care not to over dry the bead ring (bead ring appears cracked) as this will significantly decrease elution efficiency.

3.7 Elute DNA : Remove the reaction vessel from magnetic separator and add 30-40  $\mu$  L Water ( molecular biology grade ) or Tris-HCl or TE. Mix the total elution volume by pipetting 10 times to resuspend the beads and incubate at RT for 3-5minutes. Place the reaction vessel on a magnetic separator to magnetize the BeaverBeads™ DNA Select Isolation. Let sit at room temperature until the beads are completely settle to the magnet. Transfer the cleared supernatant containing size selected sample to an appropriate storage vessel.

**TIP:** The elution volume of this product can be as low as 20  $\mu$  L, but attention should be paid that the elution efficiency is related to the elution volume, the more the elution volume, the more the total amount of the nucleic acid, but the lower the concentration.



**Fig.1 Pseudogel image from the fragment analysis of DNA recovered after size selection.**

Note:Left is the Left Side Size Selection, Right is the Right Side Size Selection.

### Note

1. This product should not be frozen, dried or centrifuged. Freezing, drying and centrifugation will cause the beads to agglomerate, not easy to resuspend and disperse, and affect the chemical activity of beads surface functional group.
2. Before using this product, be sure to fully oscillate or ultrasonic to keep the beads in a uniform suspension.

- This product must be used with magnetic separation equipment.
- This product is for research use only.

**Table 1: Double-ended size selection ratio**

| DNA Size selection |     | 200~300 bp | 300~400 bp | 400~500 bp | 500~600 bp | 600~800 bp |
|--------------------|-----|------------|------------|------------|------------|------------|
| volume of beads    | 1st | 0.80×      | 0.75×      | 0.65×      | 0.60×      | 0.50×      |
|                    | 2nd | 0.21×      | 0.10×      | 0.10×      | 0.10×      | 0.10×      |

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