Genomic DNA Extraction Kit for Tissues (HMW, Magnetic Beads)

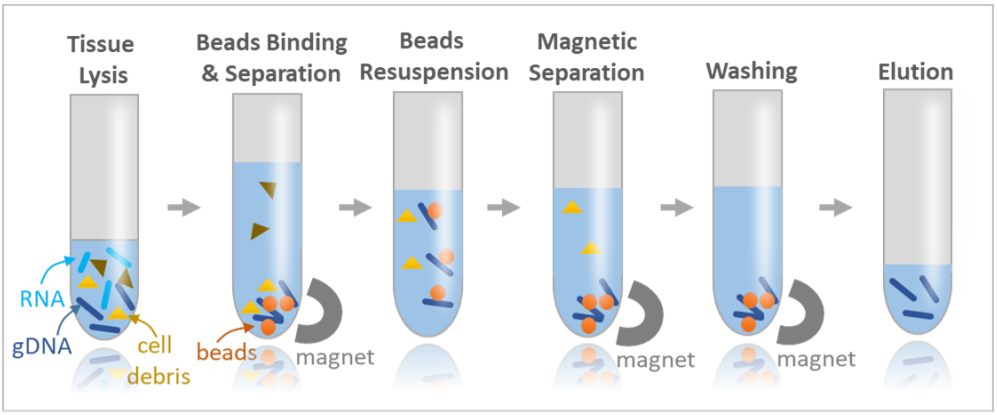
Catalog No.	50016S	50016SP*	50016L	50016LP*
Preps	50 preps	50 preps	200 preps	200 preps

*With Disposable Pestle and Tube Set

Description

The **Genomic DNA Extraction Kit for Tissues (HMW, Magnetic Beads)** provides a reliable and fast process for extracting high molecular weight (HMW) genomic DNA from tissues using Solid Phase Reversible Immobilization (SPRI) magnetic beads. With our proprietary magnetic beads technology, the kit eliminates the tedious centrifuge steps for columns. The kit provides a reliable and simple approach for high-quality genomic DNA isolation with fast magnetic response time and high binding capacity.

The extracted HMW genomic DNA size ranges are dependent on the beads resuspension: 50-150 kb by tube tapping and 40-100 kb by tube vortexing. Purified DNA is recovered at high yield and high purity without RNA contamination. The typical purity ratios of A260/A280 are around 1.8-2.0, and A260/A230 are around 2.2-2.5. Purified HMW genomic DNA is suitable for applications such as long-read sequencing, linked-read genome assembly, long range PCR, optical mapping, and other general applications.



Tissues are homogenized and lysed, then mixed with beads to bind genomic DNA. The samples are mixed with a buffer and after washing steps, genomic DNA is eluted in Elution Buffer. The isolated genomic DNA with the magnetic beads is free of contamination such as RNA, proteins, salts, and other impurities.

Features

- High molecular weight DNA: 50 kb to 150 kb
- High purity
- Simple magnetic beads method
  - No centrifuge needed
  - No column needed
  - No vacuum needed

Specifications	
Technology	Magnetic beads
Tissue amount	Up to 10 mg
Genomic DNA size	50-150 kb (tube tapping) 40-100 kb (tube vortexing)
Genomic DNA yield	Up to 30 ug (dependent on the tissue)
Elution volume	100 µl
O.D. 260/280	1.8-2.0
O.D. 260/230	Above 2.0

Component

Catalog No.	50016S	50016SP	50016L	50016LP
TS Buffer	7.5 ml	7.5 ml	30 ml	30 ml
Proteinase K	100 µl	100 µl	400 µl	400 µl
RNase	150 µl	150 µl	600 µl	600 µl
Lysis Buffer	12.5 ml	12.5 ml	50 ml	50 ml
gDNA Beads	7.5 ml	7.5 ml	30 ml	30 ml
RS Buffer	15 ml	15 ml	60 ml	60 ml
MB Buffer	12 ml	12 ml	48 ml	48 ml
Elution Buffer	5 ml	5 ml	20 ml	20 ml
Disposable Pestle	-	50	-	200
Disposable Pestle Tube, 1.5 ml	-	50	-	200

Storage Condition

- Store Proteinase K at -20°C, stable up to 12 months.
- Store RNase, gDNA Beads, MB Buffer at 4°C, stable up to 12 months.
- Store TS Buffer, Lysis Buffer, RS Buffer, and Elution Buffer at room temperature, stable up to 12 months.

Reagent & Equipment Needed (not provided in this kit)

- Magnetic particle concentrator
- Vortexer
- 1.5 ml tubes
- Disposable Pestle and Tube Kit (BioDynami Cat. # 50017S and 50017L): Provided for Cat.# 50016SP and 50016LP
- 80% ethanol (prepare before use)

Protocol

Part 1, Tissue lysis

1. Prepare a master mix of TS Buffer (150 µl per sample), RNase (3 µl per sample), and Proteinase K (2 µl per sample) according to the number of samples.
2. Homogenize a sample using a disposable pestle (provided with Cat.# 50016SP and 50016LP). If working with multiple samples, each sample should be processed quickly through all the sub-steps below (steps a-d).
   a) Transfer tissue (up to 10 mg) to a 1.5 ml pestle tube (provided with Cat.# 50016SP and 50016LP), and briefly spin down.
   b) Grind the tissue in the tube using the pestle. Leave the pestle in the tube.
   c) Add 150 µl of the above master mix to the tissue with a wide-bore tip. Make sure no sample materials remain on the pestle and then discard the pestle. If the sample material sticks to the pestle, transfer it into the tube by wiping the pestle tip along the tube rim. Homogenize the tissue lysate by pipetting.
   d) Put the sample in a thermal mixer or heat block (Step 3). Repeat steps a-d with the remaining samples.
3. Incubate samples in the thermal mixer at 56°C for a minimum of 45 minutes with agitation. The agitation speed at 1400-2000 rpm is recommended for most applications. Alternatively, incubate samples in a heatblock at 56°C for 1-3 hours and tap the tube from time to time to speed up tissue lysis. Samples will turn from turbid to clear or mostly clear when lysis is complete.

Part 2, Genomic DNA extraction

Note: Resuspend gDNA beads completely before use.

1. Add 250 µl of the Lysis Buffer and mix by rotating the tube for 5 min.
2. Add 150 µl of the gDNA Beads, mix by inverting the tube 8 times and put the tube on a magnet rack immediately. Incubate for 5-10 min and remove the supernatant using pipette tips. Remove any residue solution using a pipette with fine tips. DO NOT touch the beads.
3. Add 800 µl of 80% ethanol, remove samples from the magnet, resuspend the beads by vortexing briefly and gently.
4. Put samples on the magnet and discard the supernatant. Remove any residue solution using a pipette with fine tips.
5. Add 300 µl of RS Buffer, remove samples from the magnet, resuspend the beads with one of the following:
   a) Tube tapping or
   b) Tube vortexing
   Note: It may take 5 to 30 min to fully resuspend the beads. The extracted DNA size ranges are dependent on the beads resuspension: 50-150 kb by tapping and 40-10 kb by vortexing.
6. Transfer samples to new tubes.
7. Add 240 µl of the MB Buffer, mix by inverting the tube 8 times and put the tube on a magnet rack immediately. Incubate for 5 min and carefully remove the supernatant using pipette tips. DO NOT touch the beads.
8. Add 800 µl of 80% ethanol, remove samples from the magnet, resuspend the beads clumps in solution by vortexing briefly and gently. Invert the tube several times and incubate for 2 min. Make sure all the beads clumps are in the solution. Put samples on the magnet and discard the supernatant.
9. Repeat the ethanol washing (step 8) one more time.
10. Put samples on the magnet and discard the supernatant. Dry the beads for 1 min and remove any residue solution using a pipette with fine tips.
11. Add 100 µl of Elution Buffer (10 mM Tris, pH 8.0), resuspend the beads with one of the following:
    a) Tube tapping or
    b) Tube vortexing
    Note: It may take 5 to 30 min to fully resuspend the beads. The extracted DNA size ranges are dependent on the beads resuspension: 50-150 kb by tapping and 40-10 kb by vortexing. DNA can also be resuspended in TE buffer or Low TE buffer.
12. Put the samples on the magnet and transfer the supernatant (containing DNA) to new tubes.

Troubleshooting

Low DNA concentration

1. Up to 10 mg of tissue can be used. Over 10 mg of tissue will make it difficult to resuspend DNA in solution.
2. Certain types of tissue may have relatively low DNA yield.
3. All tissue should be collected at the bottom of the tube (Part 1, Tissue lysis). Make sure no tissue material sticks to the pestle or the wall of the tube.
4. Tissue lysis is critical for DNA yield. Make sure the tissue is completely lysed. Pipetting may be needed if tissue remains un-lysed and extended time may be needed for tissue lysis.
5. Mix completely after adding the Lysis Buffer.
6. Resuspend gDNA beads completely before use. The gDNA Beads is viscous and make sure to add an accurate volume of gDNA Beads.
7. Make sure the beads are fully resuspended in the buffer (step 4 and 11). Extend the time if needed.

Low O.D. 260/230

1. Discard supernatant completely when washing (step 8 and 10).
2. Remove any residue solution using a pipette with fine tips at step 10.

Quality Control

Kit components passed stringent functional quality test.

Product Use Limitation

This product is developed and sold for research purposes and *in vitro* use only. Please refer to biodynami.com for Material Safety Data Sheet of the product.

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