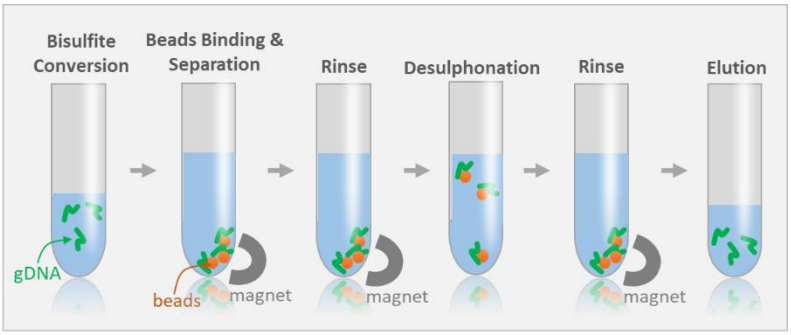
Bisulfite Conversion Kit (Magnetic Beads)

Catalog No.	15701S	15701L
Reactions	24	48

Description

The **Bisulfite Conversion Kit (Magnetic Beads)** was developed for the detection of genomic DNA methylation using a simple bisulfite conversion approach. Bisulfite conversion is a standard technique for DNA methylation analysis. The conversion can distinguish methylated cytosines from unmethylated cytosines at single base resolution of genomic DNA.

Bisulfite conversion converts unmethylated cytosines to uracils using sodium bisulfite, while methylated cytosines (5-mC) remain unchanged. First, genomic DNA is denatured to single-stranded DNA and treated with sodium bisulfite. Sodium bisulfite selectively converts unmethylated cytosines into uracils through deamination, while the methylated cytosines (including both 5-methylcytosine and 5-hydroxymethylcytosine) are not changed. The conversion is typically followed by PCR, in which uracils are further converted to thymines. As a result, all unmethylated cytosines are converted into thymines, and all methylated cytosines remain cytosines. The conversion difference of methylated and unmethylated cytosines can be detected and identified by comparison to the original genome.



Our **Bisulfite Conversion Kit (Magnetic Beads)** combines DNA denaturation and bisulfite conversion into one step. Solid Phase Reversible Immobilization (SPRI) beads is used for desulphonation and purification. The beads are paramagnetic particles coated with carboxyl groups that can reversibly bind to DNA fragments and are effective and easy for DNA purification. Our magnetic beads also allows the elution of converted DNA in a small volume of solution without yield loss.

Popular downstream applications for analyzing DNA methylation include PCR, methylation-specific PCR (MS-PCR), COBRA, pyrosequencing, and Next Generation Sequencing (NGS).

Feature

- High conversion efficiency (>99%)
- Input DNA range: 1-1000 ng
- Ready-to-use reagents
- Low elution volume without yield loss
- Beads-based technology
    - No centrifuge
    - No column
    - No vacuum

Component

Catalog No.	15701S	15701L
Conversion Buffer	1.2 ml X3	1.2 ml X6
Conversion Beads	7.5 ml	15 ml
Desulphonation Buffer	2.5 ml	5 ml
Elution Buffer	1.5 ml	3 ml

Storage Condition

- Store at -20°C: Conversion Buffer (protect from light), stable up to 12 months
- Store at 4°C: Conversion Beads, Desulphonation Buffer, and Elution Buffer, stable up to 12 months.

Reagent & Equipment Needed (not provided in this reagent)

- Thermal cycler with heated lid
- Magnetic Beads Separation Rack
- 1.5 ml tubes
- 100% ethanol
- 80% ethanol (prepare before use)

Protocol

Note:

- Invert or shake the beads to thoroughly resuspend the beads.
- Prep of **Desulphonation & Ethanol Mix** by mixing the following (per sample) before use:

Desulphonation Buffer	88 µl
100% Ethanol	352 µl
Total	440 µl

1) Mix the following in a PCR plate. **Note**: Make sure there are no droplets on the side of the wells.

DNA	20 µl (1-1000 ng)
Conversion Buffer	130 µl
Total	150 µl

2) Put the PCR plate on a thermal cycler and start the following condition (with a heating lid):

Step	Temperature	Time	Cycles
Initial Denaturation	95°C	5 minutes	1
Denaturation	95°C	5 minutes	3 cycles
Conversion	55°C	30 minutes	

3) While the samples are on the thermal cycler, transfer 300 µl of Conversion Beads to 1.5 ml tubes and let the beads reach room temperature. **Note**: Slow pipetting of the viscous beads is needed for precise aliquots.
4) Transfer the DNA samples from the PCR plate to the tubes containing Conversion Beads, mix thoroughly by vortexing. Incubate for 30 min.
5) Load the tubes on a magnet, incubate for 5 min, and remove the supernatant carefully using a pipet. Wait for 1 min, remove residual ethanol using fine tips without disturbing the beads.
6) While the tube is on the magnet, add 800 µl of 80% ethanol without disturbing the beads, invert the tubes several times, incubate for 3 min, and discard the supernatant carefully.
7) Add 400 µl of the newly prepared Desulphonation & Ethanol Mix, mix thoroughly by vortexing for 30 seconds, incubate for 15 min.
8) Load the tubes on a magnet, incubate for 1 min, and discard the supernatant carefully. Wait for 1 min, remove residual ethanol using fine tips without disturbing the beads.
9) While the tubes are on the magnet, add 750 µl of 80% ethanol without disturbing the beads, invert the tubes several times, incubate for 2 min, and discard the supernatant carefully. Repeat this step one more time.
10) Air-dry the beads on the magnet for 1 min, remove residual ethanol using fine tips without disturbing the beads. Continue Air-dry for 7 min. **Note**: Do not over-dry the beads. Go to step 11 immediately if beads cracks are observed.
11) Remove the tubes from the magnet and resuspend the beads in Elution Buffer (Tris-HCl 10 mM, pH 8.0), low TE buffer, TE buffer, or water. **Note**: Resuspending the beads in less than 20 µl may reduce the yield.
12) Load the tubes on the magnet, incubate for 1 min, and transfer the supernatants (containing samples) to new tubes without disturbing the beads.

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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