

GDSNext Ligation Module

Instruction for Use

【Product Name】

GDSNext Ligation Module

【Cat. No./Spec.】

K026-A (24 rxns); K026-B (96 rxns)

【Product Description】

The GDSNext End Preparation Module is an end-repair/dA-tailing module for NGS library preparation, and has been optimized to convert 500 pg-1 µg of fragmented DNA to repaired DNA having 5' phosphorylated, 3' dA-tailed ends.

The GDSNext Ligation Module has been optimized to ligate efficiently DNA adaptors compatible with Illumina sequencing to end-repaired, dA-tailed DNA fragments. The module is optimized for use with the GDSNext End Preparation Module (#K025). This module enables high yield preparation of high quality libraries from 500 pg to 1 µg of input DNA.

【Components】

Component	K026-A (24 rxns)	K026-B (96 rxns)
GDSNext DNA Ligase	120 µL	480 µL
GDSNext Ligation Buffer	600 µL	4×600 µL

【Storage Condition & Shelf Life】

All reagents should be stored at -20°C. Ligation Buffer is normal for crystals to precipitate at low temperatures, it should be balanced to room temperature before use. The product is valid for 12 months.

【Application】

Adapter ligation in NGS library preparation.

【Protocol】

Protocol for Use with GDSNext End Preparation Module (#K025)

Starting material: 500 pg-1 µg DNA fragments that has been end repaired and dA-Tailed using the GDSNext End Preparation Module (#K025). The Adapter should be selected and diluted to an appropriate concentration with 0.1X TE before ligation.

Table 1 Recommended Use Concentrations of Adapter

DNA Input	Recommended Conc. for Adapter	Adapter:Insert Mole Ratio*	GDS Adapter Dilution Degrees**
1 µg	10 µM	10:1	No dilution
500 ng	10 µM	20:1	No dilution
250 ng	10 µM	40:1	No dilution
100 ng	7.5 µM	100:1	3:4
50 ng	5 µM	200:1	1:2
25 ng	2.5 µM	200:1	1:4
1 ng	1 µM	200:1	1:10

* Adapter:Insert mole ratio refers to the ratio of the Adapter molar number from other sources to the Input DNA molar number, which can be roughly calculated by referring to the following formula:

Input DNA number (pmol)≈Input DNA mass (ng)/[0.66×Input DNA average length (bp)]

**The quality and concentration of the Adapter greatly affect the output of the library, especially for low input libraries. The Adapter from a high-quality source should be selected and diluted to an appropriate concentration with 0.1×TE before ligation. For immediate use, ensure that each sample addition is a fixed 5 µL, avoid sample addition errors, and try to avoid repeated freeze-thaw.

1. Add the following components to End Prep Reaction Mixture:

Reagents	Volume
End Prep Reaction Mixture	65 µL
GDSNext DNA Ligase	5 µL
GDSNext Ligation Buffer ^a	25 µL
Adapter ^b	5 µL
Total volume	100 µL

^a Prior to the addition of the reaction, mix GDSNext Ligation Buffer by pipetting up and down several times to completely dissolve the white crystal solidified at low temperature and restore it to normal temperature.

^b GDSBio Adapter Cat. No. #K002, #K003.

Note: All components are ready to use, avoid mixing in advance.

2. Set a 100 µl or 200 µl pipette to 80 µl and then pipette the entire volume up and down at least 10 times to mix thoroughly. Perform a quick spin to collect all liquid from the sides of the tube.

Note: It is important to mix well.

3. Place in a thermal cycler, and run the following program:

Temperature	Time

20°C	15 min
4°C	hold

4. DNA is now ready for size selection or cleanup.

【Library Preparation Module】

GDSBio offers the following DNA and RNA library construction modules that can be used in combination for high-quality library preparation:

Module	Product Name	Cat. No./Spec.
cDNA First Strand Synthesis	GDSNext RNA First Strand Synthesis Module	K020-A/24 rxns K020-B/96 rxns
Directional cDNA Second Strand Synthesis	GDSNext Directional RNA Second Strand Synthesis Module	K021-A/24 rxns K021-B/96 rxns
Non-Directional cDNA Second Strand Synthesis	GDSNext Non-Directional RNA Second Strand Synthesis Module	K022-A/20 rxns K022-B/100 rxns
Fragmentation & End Repair	GDSNext Fragmentation & End Prep Module	K023-A/24 rxns K023-B/96 rxns
Fragmentation	GDSNext dsDNA Fragmentase	K024-A/50 rxns K024-B/250 rxns
End Repair/dA-Tailing	GDSNext End Preparation Module	K025-A/24 rxns K025-B/96 rxns
Adapter Ligation	GDSNext Ligation Module	K026-A/24 rxns K026-B/96 rxns
Amplification	HIFI Library PCR Master Mix	K007-A/40 rxns K007-B/400 rxns K007-C/2000 rxns
Cleanup/Size Selection	GDSPure DNA Selection Magbeads	NC1011/5 mL NC1012/60 mL NC1013/450 mL

This product is for research use only.