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WGS Ligase

Instructions for Use

Product Number

L6030-W-F and L6030-W-L

Product Description

The WGS ligase provides optimized adaptor ligation chemistry for library construction optimized to follow Enzymatic's 5X WGS Fragmentation Mix (Y9410L or Y9410F).

Components

Part Number	L6030-W-L	L6030-W-F
Number of reactions	**24 Reactions**	**8 Reactions**
WGS Ligase	240 µL	80 µl
5X Rapid Ligase Buffer	500 µL	500 µL

Reagents Not Supplied

1) 5X WGS Fragmentation Mix (*Y9410L or Y9410F can be purchased from Enzymatics)*
2) DNA Adapters
3) AMPure® XP Beads
4) Nuclease free water
5) 80% Ethanol
6) 10 mM Tris-HCl, pH 8.0

General Precautions

- Use good laboratory practices to minimize cross-contamination of nucleic acid products.
- Always use PCR tubes, microfuge tubes and pipette tips that are certified sterile, DNase- and RNase-free.
- Before starting, wipe down work area and pipettes with an RNase and DNA cleaning product such as RNase Away® (Molecular BioProducts, Inc. San Diego, CA).
- For consistent library amplification, ensure the thermal cycler used in this protocol is in good working order and has been calibrated to within the manufacturer's specifications.
- Read the entire protocol before beginning. Take note of stopping points where samples can be frozen at -20°C and plan your workflow accordingly.

Storage and Handling

All reagents should be stored at -25°C and -15°C

Protocol

WGS Ligase

1. Transfer Y µl of DNA adapter* into the PCR tube with 50 µl of A-tailed DNA from 5X WGS Fragmentation. Mix gently by pipetting and cool on ice.

   * Note: DNA adapters are not included. Follow supplier's recommendation for adapter concentration and usage condition. Generally, we recommend an adapter to insert molar ratio from 25:1 to 200:1 based on the amount of input DNA (1µg – 1ng) and size of the targeted DNA fragment, in order to achieve optimal ligation efficiency.

2. Prepare the following ligation reaction master mix (per DNA sample) in a separate tube on ice and mix well by pipetting. It can be scaled as needed for the desired number of samples (10% extra volume added to compensate for the pipetting loss when preparing the master mix for multiple samples).

	1 reaction (µl)
5X Ligation Buffer	20
DNA ligase	10
Nuclease-free H_2O	(20 - Y)
Total	(50 – Y)

3. Add (50 – Y) µl of the ligation master mix to the sample from step 1 and mix well by pipetting. Incubate the ligation reaction at 20°C for 15 min.

   IMPORTANT: Do not use a thermocycler with a heated lid.

4. Proceed immediately to adapter ligation cleanup using 0.8X (80 µl) AMPure® XP beads.

   a) Equilibrate AMPure® XP beads to room temperature (RT) for 20 min.
   b) Add 80 µl of thoroughly vortexed AMPure® XP beads slurry to the ligation sample from step 3 and mix well by pipetting.
   c) Incubate the mixture for 5 min at RT. Pellet the beads on a magnetic stand (*e.g.*, DynaMag®) and carefully discard the supernatant.
   d) Wash the beads with 200 µl of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once.
   e) Air-dry the beads on the magnetic stand for 10 min or until the beads are dry. Over-drying of beads may result in lower DNA recovery.

f) Resuspend the dried beads in 52.5 µl of 10 mM Tris-HCl, pH 8.0. Pellet the beads on the magnetic stand. Carefully transfer 50 µl of supernatant into a new tube.

5. If no size selection is required, perform a second purification using 1X (50 µl) AMPure® XP beads. Elute DNA in 28 µl of 10mM Tris-HCl, pH 8.0. Pellet beads and carefully collect 25.5 µl of purified DNA sample for library amplification. If size selection is required, please use your choice of method and follow the corresponding protocols. Alternatively, if library amplification is not intended, elute DNA in 12.5 µl of 10mM Tris-HCl, pH 8.0 after second 1X AMPure® XP beads purification. Pellet beads and carefully collect 10 µl of purified DNA sample. If not proceeding immediately, the sample can be stored at -20°C.

Quality Control

All kit components are subjected to stringent quality control tests, are free of contaminating exo- and endonuclease activities and meet strict requirements with respect to DNA contamination. Detailed product information for individual kit components is available upon request, please contact tech@enzymatics.com.

Limitations of Use

This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. SDS sheets relevant to this product are available upon request.

For more information please visit www.enzymatics.com

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Enzymatics Inc.
100 Cummings Center, Suite 407J
Beverly, MA 01915
Tel: 888.927.7027
Fax: 978.867.5724