Glycerol-Free T4 DNA Ligase (HC)

Shipping:	Blue Ice
Catalog number:	MDX200
Batch No.:	See vial
Concentration:	3300 U/µL (50 Weiss unit/µL)

Store at –20°C

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Storage and stability:
Glycerol-Free T4 DNA Ligase (HC) is shipped on Blue Ice and should be stored at -20°C upon receipt. Repeated freeze/thaw cycles should be avoided.

Expiry
When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

Safety precautions:
Please refer to the material safety data sheet for further information.

Quality control specifications:
Glycerol-Free T4 DNA Ligase (HC) activity is assayed by testing the efficiency of relegation of digested lambda DNA using PCR. Glycerol-Free T4 DNA Ligase (HC) is tested for purity, exo- and endonuclease contamination prior to release.

Notes:
This reagent has been manufactured under 13485 Quality Management System and is suitable for further manufacturing use.

Description

Meridian Glycerol-Free T4 DNA Ligase (HC) is designed for the ligation of both blunt and sticky-ended fragments in just 15 and 10 minutes respectively at room temperature (25°C). Glycerol-Free T4 DNA Ligase (HC) can be used to perform linker ligation, re-ligation of linearized plasmids and ligation of double-stranded oligonucleotides into vectors. This enzyme enables rapid ligation of up to 100ng of fragments from most sources including PCR fragments, plasmids, cosmids, genomic, phage and viral DNA into prokaryotic or eukaryotic plasmid vectors and bacteriophage lambda vectors.

Components

Product Name	660,000 U
Glycerol-Free T4 DNA Ligase (HC)	1x200 µL

Ligation Protocol:

1) Assemble the reaction in a microcentrifuge tube at room temperature in the order outlined below:
   a) Combine the vector and the insert in the appropriate ratio to make up no more than 100 ng of DNA.
   b) Adjust volume to 15 µL with ddH_2O.
   c) Add 0.1—1 µL of Glycerol-Free T4 DNA Ligase (HC). If needed, dilute the Ligase in 1x Reaction Buffer.
   d) Add 4 µL of MDX204 Lyo-Ready™ Ligase Reaction Buffer, 5x (always vortex before use).

2) Mix thoroughly by pipetting.

3) **Incubate at room temperature for 10 minutes for cohesive ends, or for 15 minutes for blunt ends.**

4) *Optional:* run 2.5-5 µL of ligation mixture on to an agarose gel to check ligation efficiency before subjecting the DNA to transformation reactions.

5) Product is ready for transformation (or storage at -20°C).
   No heat inactivation required.

Notes

- Always vortex MDX204 Lyo-Ready™ Ligase Reaction Buffer, 5x before use.
- Avoid multiple freeze/thawing of enzyme and buffer.
- As Mg/ATP ratio is crucial for successful reaction, avoid the use of DNA solutions with a concentration of EDTA higher than 0.1mM.

General Considerations:

1. **Preparation of Vector and Insert DNA molecules:** For ligation to occur efficiently the ends of the DNA molecules must be compatible. Prepare the Vector and insert DNA molecules obtained by restriction digest, PCR amplification, or other physical/enzymatic methods. In order to separate the vector and insert from other contaminating molecules, the DNA to be ligated should be purified, by using electrophoretic, physical or organic extraction followed by ethanol precipitation. After purification, the DNA should be quantified.
2. **Dephosphorylation of Vector DNA molecules:** When blunt-ended ligations are carried out, it may be beneficial to dephosphorylate the vector (remove the 5′-phosphate groups) to prevent self-ligation.
3. **Cloning of PCR Amplification Products:** A simple way of preparing the fragments for ligation is by incorporating the restriction enzyme sites near the 5′-end of the PCR primers, and digesting following amplification. Alternatively, for use with a dephosphorylated vector, phosphate groups can be added to either the 5′-end of primers used in the PCR, or to the amplification products themselves.
4. **Ratio of Vector DNA:Insert DNA:** The ideal vector-insert, or phage-insert ratio may be determined empirically, but is generally in the molar range of 1:3 for vector:insert ligations, or 8:1 for vector arm:insert phage ligations. For the first cloning of PCR-product, the ratio recommended for vector-insert ligation is 1/10 to 1/100.
5. **ATP Concentration:** ATP is present in MDX204 Lyo-Ready™ Ligase Reaction Buffer, 5x and is optimised to favor best performance of T4 DNA Ligase.
6. **Enzymatic Treatment of Ligation Reactions:** Following ligation, DNA molecules may be treated with any enzyme without danger of interference from MDX204 Lyo-Ready™ Ligase Reaction Buffer, 5x.

Related Products	Cat. No.
Lyo-Ready™ Ligase Reaction Buffer, 5x	MDX204

Lyophilization & Post-Lyophilization User Guideline

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The guidelines in this document can help users avoid problems in lyophilization. For storage and stability, expiry and general handling of these product pre-lyophilization, please refer to the individual Product Handling Guides.

Safety precautions:

Read and understand the SDS (Safety Data Sheets) before handling the reagents. Copies of these SDSs are available on our website or upon request.

There are several advantages for lyophilization, including room temperature shipping and storage, extended shelf-life and increased flexibility in sample volume. In order to be compatible with lyophilization however, enzyme preparations must be glycerol-free and include specialized lyophilization-excipients that preserve the mixture as it is exposed to various lyophilization conditions including freezing, temperature ramps, vacuum and dehydration. An ideal lyophilization formulation should stabilize an enzyme in a freeze-dried format and allow very fast rehydration and reactivation of the enzyme preparations, without impacting its performance post rehydration.

Lyophilization Protocol

Assemble the reaction in a microcentrifuge tube at room temperature in the order outlined below:

1. Combine reagents in the order shown in Table 1. The volumes shown are sufficient for 20 x 20 µL reactions.
2. Vortex thoroughly and pulse-spin in a microcentrifuge.
3. Keep the mixture on ice until ready to dispense.
4. Aliquot 5 µL of the mixture into an appropriate vessel and spin down.
5. Refer to the « MDX200 Lyophilization and Post-Lyophilization User Guideline » for recommended cycling conditions for lyophilization.
6. Seal and store lyophilized material at room temperature until ready for use.
7. Add template DNA to each tube containing lyophilized material and add nuclease-free water up to 20 µL.
8. Briefly vortex and spin down in a microcentrifuge
9. Incubate at room temperature for 10 minutes for cohesive ends, or for 15 minutes for blunt ends. Incubation times can be extended if necessary.

Table 1

Reagent Name	Volume	Working Concentration
Lyo-Ready™ Ligase Reaction Buffer, 5x	80 µL	4x
Glycerol-Free T4 DNA Ligase (HC)	2 - 20 µL	66 - 660 U/µL
Water	Up to 100 µL	

Lyophilization

- The lyophilization cycle protocol in table 1 is suitable for lyophilization of the Glycerol-Free T4 DNA Ligase (HC) added to MDX204 Lyo-Ready™ Ligase Reaction Buffer, 5x in standard reaction tubes and plates. These parameters are provided as a guidance only and should be optimized to different user formats and systems.
- An annealing step can be added during the freezing step to assist crystallization of amorphous material.
- Combined primary and secondary drying time can be extended up to 24 hours.
- For product containing excipients, there should be no need to add any further excipients to assist lyophilization.

Table 2. Lyophilization guidelines

Step	Temperature	Time	Description
Freezing	+4 °C	10 min	Hold
	-45 °C	1.0 °C/min	Ramp
Primary Drying	-45 °C	180 min	Hold
	-40 °C	0.5 °C/min	Ramp
	-40 °C	720 min	Hold
Secondary Drying	+25 °C	0.5 °C/min	Ramp
	+25 °C	240 min	Hold

Post-Lyophilization

For maximum shelf-life, we suggest packaging lyophilized material under inert gas conditions (e.g. nitrogen or argon) and insert a desiccant sachet to improve stability. Pouches should be heat-sealed and labelled.