

# Control Reaction for T4 DNA Ligase Activity

This protocol is for the Control Reaction for T4 DNA Ligase Activity

## Control Reaction for T4 DNA Ligase Activity

Unsuccessful ligation may be a result of inactivated ligase or inhibition of ligation reaction by impurities in sample DNA. To assess the activity of T4 DNA ligase we recommend to perform ligation reaction with control DNA, e.g. Lambda DNA/HindIII DNA Marker.

1. Prepare the control ligation mixture:

<b>Lambda DNA/HindIII DNA Marker (#SM0101)</b>	1 $\mu$ l (0.5 $\mu$ g)
<b>10X T4 DNA Ligase Buffer</b>	2 $\mu$ l
<b>T4 DNA Ligase</b>	1 u
<b>Water, nuclease-free</b>	to 20 $\mu$ l
<b>Total volume</b>	<b>to 20 <math>\mu</math>l</b>

2. Mix thoroughly, spin briefly to collect all drops and incubate at 22°C for 10 min.
3. Prepare the loading mixture:

<b>Ligation reaction product</b>	10 $\mu$ l
<b>6X DNA Loading Dye &amp; SDS Solution</b>	2 $\mu$ l



Figure 1. Evaluation of T<sub>4</sub> DNA Ligase activity in control experiment.

- 1 – Lambda DNA/HindIII fragments, unligated  
2 – Lambda DNA/HindIII after ligation reaction

## Interpretation of results

- Appearance of higher molecular weight bands and decreased intensity of the lower molecular weight bands indicates the active ligase.
- Unchanged band pattern after ligation indicates inactivated ligase.

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**North America**  
**Technical Services:**  
 techservice.genomics@thermofisher.com  
**Customer Services:**  
 customerservice.genomics@thermofisher.com  
 Tel 800 235 9880  
 Fax 800 292 6088

**Europe and Asia**  
**Technical Services:**  
 techservice.emea.genomics@thermofisher.com  
**Customer Services:**  
 customerservice.emea.genomics@thermofisher.com

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