

User Manual

Version 3.0

Product name: T4 RNA Ligase 1 (ssRNA Ligase)

Cat #: T4RL1-100, T4RL1-200, T4RL1-OEM, B-TR1, ATP

Description:

T4 RNA Ligase 1 catalyzes the ligation of a 5'-phosphoryl-terminated nucleic acid donor to a 3'-hydroxyl-terminated nucleic acid acceptor through the formation of a 3' -> 5' phosphodiester bond, with hydrolysis of ATP to AMP and PPi. Substrates include single-stranded RNA and DNA as well as dinucleoside pyrophosphates.

Protocol:

Ligation of an oligo to single stranded DNA (cDNA) Set up a 20 µl reaction as follows: 1 X Reaction Buffer 25% (wt/vol) PEG 8000 1 mM hexamine cobalt chloride (optional) 10~20 units T4 RNA Ligase 1 mM ATP Incubate at 25°C for 16 hours. Stop the reaction by adding 40 µl 10 mM Tris-HCl pH 8.0, 2.5 mM EDTA.

RNA Circularization

Set up a 20 µl reaction as follows: 1 X Reaction Buffer RNA (example: 10 µM 5'- [³²P]rA20, 10 µM in 5' termini) 10~20 units T4 RNA Ligase 0.5 µl RNase inhibitor (M0314, 40 u/ul) 10% PEG8000 20-50 µM ATP Incubate at 25°C for 1-2 hours. For longer oligos, overnight incubation at 16°C may improve yield. Boil for 2 minutes to terminate the reaction.

3´ End Labeling of RNA

Set up a 30 µl reaction as follows: 1 X Reaction Buffer 1mM ATP 1 µg RNA 10 % (v/v) DMSO 1 µM [³²P]pCp 20 units T4 RNA Ligase Incubate overnight at 16°C