RtcB Ligase

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M0458S 004170219021

M0458S

25 reactions **15 µM** **Lot: 0041702**

RECOMBINANT **Store at –20°C** **Exp: 2/19**

Description: RtcB Ligase from *E. coli* joins single stranded RNA with a 3´-phosphate or 2´,3´-cyclic phosphate to another RNA with a 5´-hydroxyl (1,2). Ligation requires both GTP and $MnCl_2$ and proceeds through a 3´-guanylate intermediate (3). With substrates having a 2´,3´-cyclic phosphate end, hydrolysis to a 3´-phosphate precedes 3´ end activation with GMP and ligation.

Source: RtcB Ligase is expressed as a His-tagged fusion in *E. coli*.

Applications:

- Ligate ssRNA with a 3´-phosphate or a 2´,3´-cyclic phosphate to the 5´-OH of ssRNA
- Circularization of ssRNA with compatible ends, as above
- Detection of 3´-phosphorylated or 5´-OH RNA using labeled probes

Supplied in: 50 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT and 50% glycerol

Reagents Supplied with Enzyme:

RtcB Reaction Buffer (10X)

10 mM GTP

10 mM $MnCl_2$

Reaction Conditions: 1X RtcB Reaction Buffer, supplemented with 1 mM $MnCl_2$ and 0.1 mM GTP. Incubate at 37°C.

1X RtcB Reaction Buffer:

50 mM Tris-HCl
75 mM KCl
3 mM $MgCl_2$
10 mM DTT
(pH 8.3 @ 25°C)

Typical Protocol for RNA ligation:

1. Prepare 1 mM GTP by diluting the 10 mM stock in nuclease-free water. Store at –20°C for repeated use.
2. Assemble the following reaction in a nuclease-free PCR tube on ice:

COMPONENTS	AMOUNT
3´-phosphate RNA donor	10 pmol (0.5 pmol/µl)
5´-OH RNA acceptor	10 pmol (0.5 pmol/µl)
RtcB Reaction Buffer (10X)	2 µl
1 mM GTP	2 µl
10 mM $MnCl_2$	2 µl
RtcB RNA Ligase	1 µl (15 pmol)
Nuclease-free Water	Up to 20 µl

3. Incubate at 37°C for 1 hour.
4. We recommend cleaning up your reactions before moving on to downstream applications. This can be achieved by using a spin column-based method or phenol:chloroform extraction followed by ethanol precipitation.

Notes on Use: For difficult substrates, addition of PEG 8000 up to 15% can improve ligation yields.

Quality Control Assays

Functional Test: A 20 µl reaction containing 1X RtcB Reaction Buffer, 0.1 mM GTP, 1 mM $MnCl_2$, 0.75 µM RtcB, and 0.5 µM each of a 5´ FAM-labeled, 3´-phosphorylated 17 mer RNA and a 5´-OH 30 mer RNA achieves > 80% ligation after incubation for 1 hour at 37°C as determined by capillary electrophoresis.

RNase Activity: A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 15 pmoles of RtcB Ligase is incubated at 37°C. After incubation for 16 hours, > 90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

Exonuclease Activity: A 50 µl reaction in RtcB Reaction Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and a minimum of 15 pmoles of RtcB Ligase incubated for 4 hours at 37°C releases < 0.1% of the total radioactivity.

(see other side)

CERTIFICATE OF ANALYSIS

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1 mM GTP	2 µl
10 mM $MnCl_2$	2 µl
RtcB RNA Ligase	1 µl (15 pmol)
Nuclease-free Water	Up to 20 µl

3. Incubate at 37°C for 1 hour.
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(see other side)

CERTIFICATE OF ANALYSIS

Endonuclease Activity: A 50 µl reaction in RtcB Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 15 pmoles of RtcB Ligase incubated for 4 hours at 37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

Protein Purity: RtcB Ligase is > 95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.

References:

1. Naoko, T., Shuman, S. (2011) *J. Biol. Chem.* 286 (10), 7727–7731.
2. Naoko, T. et al. (2011), *J. Biol. Chem.* 286 (50), 43134–43143.
3. Chakravarty, A, K. et al. (2012) *Proc. Natl. Acad. Sci. USA* 109(16), 6072–6077.

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