SplintR® Ligase

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M0375S 001160118011

M0375S

1,250 units **25,000 units/ml** **Lot: 0011601**
RECOMBINANT **Store at –20°C** **Exp: 1/18**

Description: SplintR Ligase, also known as PBCV-1 DNA Ligase or *Chorella* virus DNA Ligase, efficiently catalyzes the ligation of adjacent, single-stranded DNA oligonucleotides splinted by a complementary RNA strand. This previously unreported activity may enable novel approaches for characterization of miRNAs and mRNAs, including SNPs. SplintR is ideally suited for many target enrichment workflows with applications in next-generation sequencing and molecular diagnostics. The robust activity of the enzyme and its affinity for RNA-splinted DNA substrates (apparent Km = 1 nM) enable sub-nanomolar detection of unique RNA species within a complex mixture, making SplintR ligase a superior choice for demanding RNA detection technologies.

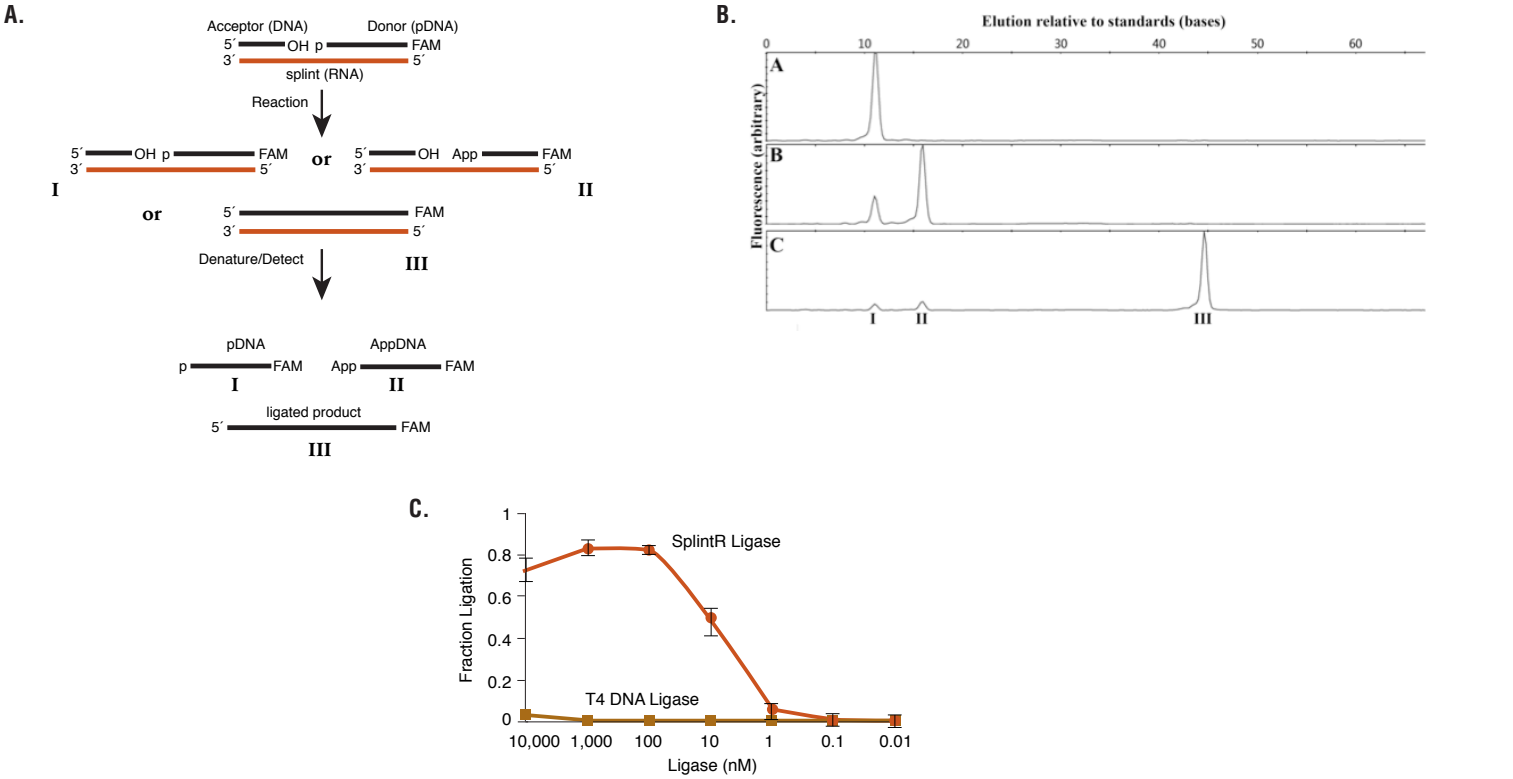
The enzyme is active over a broad range of ATP concentrations (10 µM – 1 mM) and pH (6.5–9). Optimal activity is observed using Mg^{2+} > 5 mM, and a pH between 7.5 and 8.0. The activity is enhanced at higher temperatures (up to 37°C), and by supplementation with 5 mM Mn^{2+}. The reaction is inhibited by salt concentrations in excess of 100 mM.

The enzyme tolerates all base pair combinations at the ligation junction, but is partially inhibited by dC/G and dG/C base pairs at the donor (phosphorylated) side ligation junction, particularly when the +2 base was also a C/G base pair.

Source: An *E. coli* strain that carries a recombinant gene encoding PBCV-1 DNA Ligase.

Applications:

- Ligation of ssDNA splinted by complementary RNA sequences
- Detection of RNA using ligation of DNA probes
- SNP or splice variant detection
- RASL-seq



Ligation of DNA splinted by RNA: *(A) Outline of the ligation assay: a 5´-phosphorylated, 3´-FAM labeled DNA "donor" oligonucleotide and an unmodified DNA "acceptor" oligonucleotide are annealed to a complementary RNA splint. This substrate is reacted with a ligase to form a mixture of unreacted starting material (I), adenylylated DNA (II), and ligated product (III). These products are denatured, separated by capillary electrophoresis and detected by fluorescence. (B) Ligation of the RNA-splinted substrate in SplintR Ligase Reaction Buffer for 15 minutes at 25°C with (a) no enzyme, (b) 1 µM T4 DNA Ligase and (c) 100 nM SplintR Ligase. Indicated peaks correspond to starting pDNA (I), AppDNA (II) and ligated product (III) as determined by co-elution with synthetically prepared standards. (C) The fraction of ligated product catalyzed by either SplintR Ligase or T4 DNA Ligase was analyzed by performing sets of ligations with both ligases at concentrations between 10 pM and 10 µM for 15 minutes at 25°C. SplintR Ligase is clearly much more efficient at ligation of RNA-splinted DNA than T4 DNA Ligase.*

Supplied in: 10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol and pH 7.4 @ 25°C.

Reference:

1. Lohman, G. et al. (2014) *Nucl. Acids Res.* 42, 1831–1844. doi: 10.1093/nar/gkt1032.

Reagents Supplied with Enzyme: SplintR Ligase Reaction Buffer (10X)

Reaction Conditions: 1X SplintR Ligase Reaction Buffer. Incubate at 25°C.

1X SplintR Ligase Reaction Buffer:
50 mM Tris-HCl
10 mM $MgCl_2$
1 mM ATP
10 mM DTT
pH 7.5 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme needed to ligate (to 50% completion) 2 picomoles of a tripartite FAM-labeled DNA:RNA hybrid substrate in a 20 µl reaction at 25°C in 15 minutes in 1X SplintR Ligase Reaction Buffer.

Heat Inactivation: 65°C for 20 minutes

Quality Controls

Exonuclease Activity: Incubation of a 50 µl reaction containing 125 units of SplintR Ligase with 1 µg of a mixture of single- and double-stranded [^{3}H] *E. coli* DNA for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 125 units of SplintR Ligase with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RF II as determined by agarose gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction containing 25 units of SplintR Ligase with 40 ng of fluorescein labeled RNA transcript for 4 hours at 37°C resulted in < 10% degradation of the RNA as determined by gel electrophoresis using fluorescence detection.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Usage Notes:

- SplintR Ligase is inhibited by monovalent cations. We strongly recommend ensuring these common reactants (NaCl, KCl) are kept to below 50 mM in the reaction. The enzyme is supplied in a storage buffer containing 300 mM NaCl, for storage stability. A minimum 6-fold dilution of the enzyme by addition to the reaction is recommended, with the optimal dilution being ≥ 10-fold.

(see other side)

CERTIFICATE OF ANALYSIS

- If dilution of enzyme for storage is needed, we recommend using Diluent A (NEB #B8001).
- Reactions with SplintR should be performed between 16–37°C. We recommend initial testing be performed at 25°C.
- We suggest a reaction time in the range of 10–60 minutes, with 15 minutes being ideal for many applications.
- The enzyme is supplied as a 10.5 µM solution. We suggest maintaining the enzyme below 1 µM in the reaction with a suggested range of 100 nM to 1 µM. For many applications, starting with a 2-fold excess of enzyme over ligatable ends is ideal. For example, in the experiment described in the accompanying figure a substrate concentration of 100 nM was found to be ideal. In that workflow, 250 nM enzyme gave complete ligation in 15 minutes on all sequences tested.
- If the reaction is not proceeding as efficiently as desired, we strongly recommend extending the incubation time rather than increasing the concentration of enzyme in the reaction beyond 1 µM.
- An alternative option for recalcitrant substrates is to use a low concentration of ATP. A low ATP buffer can give higher yields of ligation product for substrates that have low ligation efficiencies in the standard SplintR Ligase Reaction Buffer, such as substrates with runs of G:C base pairs at the ligation junction. We suggest 1X T4 RNA Ligase Reaction Buffer (NEB #B0216) supplemented with ATP (NEB #P0756) to a final concentration of 10 µM.
- We recommend an RNA splint of at least 20 complementary bases. We have found 10 bp of dsDNA/RNA on either side of the junction to be sufficient for all substrates tested. The splint does not have to be centered on the ligation junction, however, with as few as four bases on one side of the junction giving complete ligation for a splint with 20 bases of total complementarity, depending on substrate sequence. If regions of overlap < 10 bp are desired, some testing will be required to determine the minimum length of the ds region for your specific sequence.