Gateway™ LR Clonase™ II Enzyme Mix

Catalog Number 11791-020 and 11791-100

Doc. Part No. 11791.II.pps **Pub. No.** MAN0001032 **Rev.** A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Invitrogen™ Gateway™ LR Clonase™ II enzyme mix is a proprietary enzyme and buffer formulation containing the bacteriophage lambda recombination proteins Integrase (Int) and Excisionase (Xis), the *E. coli*-encoded protein Integration Host Factor (IHF), and reaction buffer provided in a single mix for convenient reaction set up. Gateway™ LR Clonase™ II enzyme mix catalyzes in vitro recombination between an entry clone (*att*L-flanked "gene") and an *att*R-containing destination vector to generate an *att*B-containing expression clone. Store Gateway™ LR Clonase™ II enzyme mix at –20°C (non-frost-free freezer) for up to 6 months. For long-term storage, store at –80°C.

Gateway™ technology

Gateway™ Technology is a universal cloning method that takes advantage of the site-specific recombination properties of bacteriophage lambda to provide a rapid and highly efficient way to move DNA sequences into multiple vector systems. The Gateway™ Technology is schematically represented below.

*att*B1-gene-*att*B2 × *att*P1-*ccd*B-*att*P2 ⇔ *att*L1-gene-*att*L2 × *att*R1-*ccd*B-*att*R2

(expression clone) (pDONR™) (entry clone) (destination vector)

The *att*B × *att*P reaction is mediated by Gateway™ BP Clonase™ II enzyme mix; the *att*L × *att*R reaction is mediated by Gateway™ LR Clonase™ II enzyme mix. *ccd*B is the F plasmid-encoded gene that inhibits growth of *E. coli* and "gene" represents any DNA segment of interest (e.g. PCR product, cDNA, genomic DNA).

Contents and storage

Components	Cat. no. 11791-020 (20 rxns)	Cat. no. 11791-100 (100 rxns)	Storage conditions
Gateway™ LR Clonase™ II Enzyme Mix	40 µL	200 µL	Store at –20°C (non-frost-free freezer)
Proteinase K Solution (2 µg/µL)	40 µL	200 µL	
pENTR™-gus Positive Control (50 ng/µL)	20 µL	20 µL	

Important guidelines

- pENTR™-gus is provided for use as a positive control in the LR reaction and is an entry clone containing the *Arabidopsis thaliana* β-glucuronidase (*gus*) gene. Refer to our website (**thermofisher.com**) for a map and sequence of pENTR™-gus.
- We recommend using plasmid DNA purified with the PureLink™ HQ Mini Plasmid Purification Kit (Cat. no. K2100-01). Mini-prep (alkaline lysis) DNA preparations are adequate for Gateway™ cloning reactions; however, in general, such DNA cannot be quantitated by UV absorbance due to contaminating RNA and nucleotides. Estimate concentrations by gel electrophoresis in comparison with standard DNA (e.g. DNA Mass Ladder, Cat. no. 10068-013 or 10496-016).
- For LR recombination reactions, the most efficient substrates are supercoiled *att*L-containing entry vectors and supercoiled *att*R-containing destination vectors. For large (>10 kb) entry clones or destination vectors, linearizing the entry clone or destination vector may increase the efficiency by up to 2-fold.
- To increase the number of colonies containing the desired expression clone, increase the incubation time from the recommended 1 hour to 2 hours-overnight. Longer incubations are recommended for plasmids ≥10 kb to increase the yield of colonies.
- We recommend using 50–150 ng entry clone per 10 µL reaction. Highest colony yields are typically obtained using 150 ng entry clone and 150 ng destination vector. Do not use >150 ng entry clone as you may obtain colonies containing multiple DNA molecules (often with an associated "small colony" phenotype). Using <50 ng entry clone will generate fewer colonies.

Methods

Prepare LR Reaction

LR Clonase™ II enzyme mix is supplied as a 5X solution. If you wish to scale the reaction volume, make sure the LR Clonase™ II enzyme mix is at a final concentration of 1X. For a positive control, use 100 ng (2 µL) of pENTR™-gus.

1. Add the following components to a 1.5-mL microcentrifuge tube at room temperature and mix:
   - 1–7 µL entry clone (50–150 ng)
   - 1 µL destination vector (150 ng/µL)
   - TE buffer, pH 8.0, to 8 µL
2. Thaw on ice the LR Clonase™ II enzyme mix for about 2 minutes. Vortex the LR Clonase™ II enzyme mix briefly twice (2 seconds each time).
3. To each sample (step 1), add 2 µL of LR Clonase™ II enzyme mix to the reaction and mix well by vortexing briefly twice. Microcentrifuge briefly.
4. Return LR Clonase™ II enzyme mix to –20°C or –80°C storage.
5. Incubate reactions at 25°C for 1 hour.
6. Add 1 µL of the Proteinase K solution to each sample to terminate the reaction. Vortex briefly. Incubate samples at 37°C for 10 minutes.

Transform

1. Transform 1 µL of each LR reaction into 50 µL of One Shot™ OmniMAX™ 2 T1 Phage-Resistant Cells (Cat. no. C8540-03). Incubate on ice for 30 minutes. Heat-shock cells by incubating at 42°C for 30 seconds. Add 250 µL of S.O.C. Medium and incubate at 37°C for 1 hour with shaking. Plate 20 µL and 100 µL of each transformation onto selective plates

   Note: Any competent cells with a transformation efficiency of $>1.0 \times 10^8$ transformants/µg may be used.
2. Transform 1 µL of pUC19 DNA (10 ng/mL) into 50 µL of One Shot™ OmniMAX™ 2 T1 Phage-Resistant Cells as described above. Plate 20 µL and 100 µL on LB plates containing 100 µg/mL ampicillin.

Expected results

An efficient LR recombination reaction will produce >5000 colonies if the entire LR reaction is transformed and plated.

Limited product warranty

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