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life technologies

Gateway® LR Clonase™ II Enzyme Mix

Cat. No. 11791-020 **Size: 20 reactions**
Cat. No. 11791-100 **Size: 100 reactions**
Store at -20°C (non-frost-free freezer)

Gateway® Technology

The Gateway® Technology is a universal cloning method that takes advantage of the site-specific recombination properties of bacteriophage lambda (1) 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* (2,3) and "gene" represents any DNA segment of interest (*e.g.* PCR product, cDNA, genomic DNA).

Description

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) (1), 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 -20ºC (non-frost-free freezer) for up to 6 months. For long-term storage, store at -80ºC.

Components Supplied	20 rxns	100 rxns
Gateway® LR Clonase™ II Enzyme Mix	40 µl	200 µl
Proteinase K Solution (2 µg/µl)	40 µl	200 µl
pENTR™-gus Positive Control (50 ng/µl)	20 µl	20 µl

Quality Control

LR Clonase™ II enzyme mix is functionally tested in a 1 hour recombination reaction followed by a transformation assay.

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General Recommendations and 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 (4). Refer to our Web site (www.invitrogen.com) for a map and sequence of pENTR™-gus.

- We recommend using plasmid DNA purified with the PureLink™ HQ Mini Plasmid Purification Kit (Catalog 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, Catalog 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.

Procedures

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:

Entry clone (50-150 ng)	1-7 µl
Destination vector (150 ng/µl)	1 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, above), 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.

Transformation

1. Transform 1 µl of each LR reaction into 50 µl of One Shot® OmniMAX™ 2 T1 Phage-Resistant Cells (Catalog 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.

References

1. Landy, A. (1989) *Ann. Rev. Biochem. 58*, 913.
2. Bernard, P. and Couturier, M. (1992) *J. Mol. Biol. 226*, 735.
3. Miki, T., Park, J.A., Nagao, K., Murayama, N., and Horiuchi, T. (1992) *J. Mol. Biol. 225*, 39.
4. Kertbundit, S., Greve, H.D., Deboeck, F., Montagu, M.V., and Hernalsteens, J.P. (1991) *Proc. Natl. Acad. Sci. USA, 88*, 5212.