



Gateway® LR Clonase™ Enzyme Mix

Cat. No. 11791-019

Size: 20 reactions

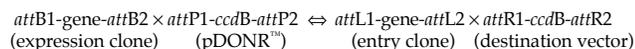
Cat. No. 11791-043

Size: 100 reactions

Store at -80°C

Gateway® Technology

Gateway® is a universal cloning technology based on the site-specific recombination properties of bacteriophage lambda (1). The Gateway® Technology provides a rapid and highly efficient way to move DNA sequences into multiple vector systems for functional analysis and protein expression, and can be schematically represented as follows:



The *attB* × *attP* reaction is mediated by Gateway® BP Clonase™ enzyme mix; the *attL* × *attR* reaction is mediated by Gateway® LR Clonase™ enzyme mix. *ccdB* 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™ enzyme mix is a proprietary enzyme formulation containing the bacteriophage lambda recombination proteins Integrase (Int) and Excisionase (Xis), and the *E. coli*-encoded protein Integration Host Factor (IHF) (1). Gateway® LR Clonase™ enzyme mix promotes *in vitro* recombination between an entry clone (*attL*-flanked “gene”) and any number of *attR*-containing destination vectors to generate *attB*-containing expression clones. “Genes” present in expression clones can be transferred back into an entry vector by mixing with an *attP* Vector (e.g., pDONR™221) and adding Gateway® BP Clonase™ enzyme mix.

Components Supplied

	20 rxns	100 rxns
Gateway® LR Clonase™ Enzyme Mix	80 µl	400 µl
5X LR Clonase™ Reaction Buffer	200 µl	1 ml
2 µg/µl Proteinase K Solution	40 µl	200 µl
50 ng/µl pENTR™-gus Positive Control	20 µl	20 µl

Part No. 11791.pps

Rev. Date: 02/26/03

This product is distributed for laboratory research only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

For technical questions about this product, call the Invitrogen Tech-Line™ U.S.A. 800 955 6288

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). 1 μ g of supercoiled pENTR[™]-gus plasmid is supplied in TE Buffer, pH 8.0 at a concentration of 50 ng/ μ l. **Note:** See our Web site (www.invitrogen.com) for a map and the sequence of pENTR[™]-gus.
- We recommend using plasmid DNA purified with the S.N.A.P.[™] MiniPrep Kit (Catalog no. K1900-01). 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, *i.e.*, DNA Mass Ladder (Cat. Nos. 10068-013 or 10496-016).
- For LR reactions, the most efficient substrates are supercoiled *attL*-containing entry vectors and supercoiled *attR*-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 or overnight. Longer incubations are recommended for plasmids \geq 10 kb to increase the yield of colonies.
- We recommend using 100-300 ng entry clone per 20 μ l reaction. Highest colony yields are typically obtained using 300 ng entry clone and 300 ng destination vector. Do not use >300 ng entry clone as you may obtain colonies containing multiple DNA molecules (often with an associated "small colony" phenotype). Using <100 ng entry clone will generate fewer colonies.

Procedures**LR Reaction**

Use the following procedure to perform an LR recombination reaction. For a positive control, use 100 ng (2 μ l) of pENTR™-gus.

1. Add the following components to a 1.5 ml microcentrifuge tube at room temperature and mix:

Entry clone (100-300 ng)	1-10 μ l
Destination vector (150 ng/ μ l)	2 μ l
5X LR Clonase™ Reaction Buffer	4 μ l
TE buffer, pH 8.0	to 16 μ l
2. Remove LR Clonase™ enzyme mix from -80°C and thaw on ice for about 2 minutes. Vortex the LR Clonase™ enzyme mix briefly twice (2 seconds each time).
3. To each sample (Step 1, above), add 4 μ l of LR Clonase™ enzyme mix to the reaction and mix well by vortexing briefly twice. Microcentrifuge briefly.
4. Return LR Clonase™ enzyme mix to -80°C storage immediately after use.
5. Incubate reactions at 25°C for 60 minutes.
6. Add 2 μ l 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 μ l of each LR reaction into 50 μ l of Library Efficiency® DH5 α ™ Competent Cells (Cat. No. 18263-012). Incubate on ice for 30 minutes. Heat-shock cells by incubating at 42°C for 30 seconds. Add 450 μ l of S.O.C. Medium and incubate at 37°C for 1 hour with shaking. Plate 20 μ l and 100 μ l of each transformation onto selective plates.
Note: Any competent cells with a transformation efficiency of $>1.0 \times 10^8$ transformants/ μ g may be used.
2. Transform 1 μ l of pUC19 DNA (10 ng/ml) into 50 μ l of Library Efficiency® DH5 α ™ Competent Cells as described above. Plate 20 μ l and 100 μ l on LB plates containing 100 μ g/ml ampicillin.

Expected Results

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

Quality Control

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

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.

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