Next Steps

Refer to the manual for the destination vector you are using for guidelines and instructions to express your recombinant protein in the appropriate system.

For more information on Gateway® MultiSite Technology, see the Gateway® Technology, MultiSite Gateway® and MultiSite Gateway® Pro manuals, available at www.invitrogen.com.

Quality Control

LR Clonase™ II Plus enzyme mix is functionally tested in a 16-hour reaction to combine 4 fragments into one DEST vector, followed by a transformation assay.

References

1. Landy, A. (1989) *Ann. Rev. Biochem. 58*, 913.

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Gateway® LR Clonase™ II Plus Enzyme Mix

Cat. No. 12538-120 **Size: 20 reactions**
Cat. No. 12538-200 **Size: 100 reactions**

MultiSite Gateway® and MultiSite Gateway® Pro

MultiSite Gateway® and MultiSite Gateway® Pro are extensions of the Gateway® site-specific recombinational cloning technology, which is based on the recombination properties of bacteriophage lambda (1). MultiSite Gateway® allows cloning of exactly three DNA fragments into pDEST R4R3, while MultiSite Gateway® Pro allows cloning of two, three, or four DNA fragments in a defined order and orientation into any pDEST vector containing *att*R1 and *att*R2 sites. Both systems provide a rapid and efficient way to recombine DNA elements into vector systems for functional analysis and protein expression. The LR recombination reaction occurs between two specific attachment sites (*att*L and *att*R) on the entry clones and the destination vector, allowing the recombination of fragments into the destination vector. The reaction is mediated by Gateway® LR Clonase™ II Plus enzyme mix.

Gateway® LR Clonase™ II Plus

Gateway® LR Clonase™ II Plus enzyme mix is a proprietary enzyme formulation specifically designed for MultiSite Gateway® and MultiSite Gateway® Pro. Gateway® LR Clonase™ II Plus enzyme mix contains the bacteriophage lambda recombination proteins Integrase (Int) and Excisionase (Xis), and 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 Plus enzyme mix promotes *in vitro* recombination between *att*L- and *att*R-flanked regions on entry clones and destination vectors to generate *att*B-containing expression clones.

Component	20 rxns	100 rxns
Gateway® LR Clonase™ II Plus Enzyme Mix	40 µl	200 µl
2 µg/µl Proteinase K Solution	40 µl	200 µl

Storage

Store Gateway® LR Clonase™ II Plus at -20°C (in a non-frost-free freezer) for up to 6 months. For long term storage, store at -80°C

Part no. 12538.pps Rev. date: 11 July 2006

General Recommendations and Guidelines

- We recommend using plasmid DNA purified with the PureLink™ HiPure Plasmid Midiprep Kit (Catalog no. K2100-04). Mini-prep (alkaline lysis) DNA preparations are **not recommended** for MultiSite Gateway® cloning reactions. DNA cannot be quantitated by UV absorbance due to contaminating RNA and nucleotides, estimate concentration by gel electrophoresis (*e.g.*, DNA Mass Ladder, Cat. no. 10068-013 or 10496-016).
- For LR reactions, supercoiled entry vectors and destination vectors provide efficient substrates.
- For large (>10 kb) entry clones or destination vectors, linearizing the entry clone or destination vector may increase the efficiency by up to two fold.

To Convert fmoles to nanograms (ng)

$$ng = (x\ \text{fmol})(N)\left(\frac{660\ fg}{fmol}\right)\left(\frac{1\ ng}{10^6\ fg}\right)$$

where N is the size of the DNA in base pairs, and *x* is the number of fmoles

MultiSite Gateway® and MultiSite Gateway® Pro LR Reaction Mixture

For multi-fragment (*i.e.* 2-, 3-, or 4-fragment recombination) reactions, use an equimolar amount of each entry clone. We recommend 10 fmol of **each** entry clone and 20 fmol of DEST vector per 10 µl reaction.

Note: If you are using MultiSite Gateway®, you can only recombine three entry clones into pDEST R4R3. If you are using MultiSite Gateway® Pro, you can recombine up to four entry clones into any pDEST vector containing *att*R1 and *att*R2 sites.

Add the following components to a 1.5-ml microcentrifuge tube at room temperature and mix. Use this reaction mixture in the **Procedure** on the next page.

Entry clones (10 fmoles)	1-7 µl total*
Destination vector (20 fmoles)	1 µl
1X TE buffer, pH 8.0	to 8 µl

*All entry clones (two, three or four, depending on the type of reaction) must be included. The total of all entry clones combined should not exceed 7 µl.

LR Reaction Procedure

1. Remove LR Clonase™ II Plus enzyme mix from freezer and thaw on ice for about 2 minutes. Vortex the enzyme mix briefly twice (2 seconds each).
2. To each MultiSite or MultiSite Pro LR reaction mixture, add 2 µl of LR Clonase™ II Plus and mix well by vortexing briefly twice. Microcentrifuge briefly.
3. Return enzyme mix to freezer immediately after use. The enzyme mix can be stored at -20°C for up to 6 months or at -80°C for long-term storage.
4. Incubate recombination reaction at 25°C for 16 hours.
5. 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. For 2- or 3-fragment recombination reactions, add 2 µl to 50 µl of One Shot® Mach1™ T1^R Chemically Competent *E. coli* and incubate on ice for 30 minutes.
2. For 4-fragment recombination reactions, add 4 µl to 50 µl of One Shot® Mach1™ T1^R Chemically Competent *E. coli* and incubate on ice for 30 minutes.
3. Heat-shock cells by incubating at 42°C for 30 seconds.
4. Immediately transfer the tubes to ice for 2 minutes.
5. Add 250 µl of S.O.C. medium and incubate at 37°C for 1 hour with shaking at 225 RPM.
6. For 2-fragment recombination reactions, dilute 1:10 in S.O.C. medium and plate 50 µl and 100 µl of each transformation on prewarmed LB plates containing 50-100 µg/ml antibiotic of choice, invert and incubate overnight at 37°C. For 3-fragment recombination reactions, plate 50 µl and 100 µl of each transformation as above. For 4-fragment recombination reactions, spin at 6,000 rpm, remove 180 µl of supernatant and gently resuspend the pellet in the residual media. Plate entire reaction as above.

Typical Numbers of Colonies (per 10 µl reaction):

2-fragment recombination reaction:	2,000-15,000
3-fragment recombination reaction:	1,000-5,000
4-fragment recombination reaction:	50-500