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Gateway® LR Clonase™ II Enzyme Mix for BaculoDirect™ Kits

Cat. No. 11791-023 **Size: 40 µl (10 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 to provide a rapid and highly efficient way to move DNA sequences into multiple vector systems. Using the BaculoDirect™ System, you will recombine an entry clone (containing your gene of interest and *att*L sites) with the BaculoDirect™ Linear DNA containing *att*R sites to produce an *att*B site-containing expression virus. This reaction is catalyzed by LR Clonase Enzyme Mix for BaculoDirect™ Kits, as diagrammed below:

Description

Gateway® LR Clonase™ II Enzyme Mix for BaculoDirect™ Kits 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.

Quality Control

LR Clonase™ II Enzyme Mix for BaculoDirect™ Kits is functionally tested in a 1 hour recombination reaction followed by a transformation assay. The Enzyme Mix is also tested for microbial contamination.

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For technical support, contact tech_service@invitrogen.com.

LR Clonase™ II Enzyme Mix for BaculoDirect™ Kits

LR Clonase™ II Enzyme Mix for BaculoDirect™ Kits combines the proprietary enzyme formulation and 5X LR Clonase™ Reaction Buffer. Use the protocol provided on the next page or provided in the BaculoDirect™ Kit manual to perform the LR reaction.

Note: For the LR recombination reaction using the BaculoDirect™ Expression System, use LR Clonase™ II Enzyme Mix for BaculoDirect™ Kits only. Do NOT substitute LR Clonase™ or LR Clonase™ II from other kits.

Materials Needed

You will need the following items on hand before beginning:

- Purified plasmid DNA of your entry clone (50-150 ng/µl in TE buffer, pH 8.0). See the BaculoDirect™ Expression System manual for details.
- BaculoDirect™ Linear DNA (300 ng/vial; provided with the BaculoDirect™ Expression and Transfection Kits)
- LR Clonase™ II Enzyme Mix for BaculoDirect™ Kits (Keep at -20°C until use)
- Optional: pENTR™/CAT control plasmid (100 ng/µl; provided with the BaculoDirect™ Expression and Transfection Kits)
- 1X TE Buffer, pH 8.0 (10 mM Tris-HCl, pH 8.0; 1 mM EDTA)
- 25°C water bath

LR Reaction Protocol

Perform Steps 1-4 in a **sterile laminar flow hood** to reduce the chances of contamination.

1. To set up your sample add the following components **directly** to the BaculoDirect™ Linear DNA vial containing 10 µl (300 ng) of DNA at room temperature and mix the contents. **Do not vortex or pipette up and down as this will shear the baculovirus DNA and reduce transfection efficiency.**

Component	Sample
BaculoDirect™ Linear DNA	10 µl (in vial)
Entry clone (100-300 ng/reaction)	1-2 µl
1X TE Buffer, pH 8.0	4-5 µl
Total volume	16 µl

   Note: To include a positive control, include a reaction using the plasmid pENTR™/CAT supplied with the BaculoDirect™ Kit in place of the Entry Clone. For a negative control, include a reaction that substitutes 4 µl of 1X TE Buffer, pH 8.0 for the enzyme mix (see step 4).

2. Remove the LR Clonase™ II Enzyme Mix for BaculoDirect™ Kits from -20°C and thaw on ice (~ 2 minutes).
3. Vortex the LR Clonase™ II Enzyme Mix for BaculoDirect™ Kits briefly twice (2 seconds each time).
4. To each sample above, add 4 µl of LR Clonase™ II Enzyme Mix for BaculoDirect™ Kits or 4 µl of 1X TE Buffer, pH 8.0 (if preparing a negative control) for a total reaction volume of 20 µl.
5. Mix well by tapping the tube several times. **Do not vortex or pipette up and down as this will shear the baculovirus DNA and reduce transfection efficiency.**
6. Incubate the reactions at 25°C for 1 hour.
7. Once the LR reaction is completed, you are ready to transfect the recombinant baculovirus DNA into insect cells. After incubation, you may analyze the LR reaction by performing PCR. Refer to the BaculoDirect™ Expression System Manual for detailed instructions.

References

1. Landy, A. (1989) *Ann. Rev. Biochem. 58*, 913.
2. Ptashne, M. (1992) A Genetic Switch: Phage (Lambda) and Higher Organisms (Cambridge MA: Cell Press)
3. Weisberg, R.A., and Landy, A (1983) Site-Specific Recombination in Phage Lambda. In *Lambda II*, R.W. Hendrix, J.W. Roberts, F.W. Stahl and R.A. Weisberg, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Press)

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