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Gateway® BP Clonase™ Enzyme Mix

Cat. No. 11789-013 **Size: 20 reactions**
Cat. No. 11789-021 **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:

*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™ enzyme mix; the *att*L × *att*R reaction is mediated by Gateway® LR Clonase™ 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® BP Clonase™ enzyme mix is a proprietary enzyme formulation containing the bacteriophage lambda recombination protein Integrase (Int) and the *E. coli*-encoded protein Integration Host Factor (IHF) (1). Gateway® BP Clonase™ enzyme mix promotes *in vitro* recombination between an *att*B-PCR product (or *att*B-containing expression clone) and an *att*P-containing donor (*i.e.,* pDONR™) vector to generate *att*L-containing entry clones. "Genes" in entry clones can then be transferred into any number of *att*R-containing destination vectors using Gateway® LR Clonase™ enzyme mix.

Components Supplied	20 rxns	100 rxns
Gateway® BP Clonase™ Enzyme Mix	80 µl	400 µl
5X BP Clonase™ Reaction Buffer	200 µl	1 ml
2 µg/µl Proteinase K Solution	40 µl	200 µl
30% PEG 8000/30 mM $MgCl_2$ Solution	1 ml	5 ml
50 ng/µl pEXP7-tet Positive Control	20 µl	20 µl

Part No. 11789.pps Rev. Date: 02/19/03

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

- pEXP7-tet is provided for use as a positive control in the BP reaction, and is an ~1.4 kb linear fragment containing *att*B sites flanking the tetracycline resistance gene and its promoter.
- For *att*B-containing expression clones, we recommend using plasmid DNA purified with the S.N.A.P.™ MiniPrep Kit (Cat. No. K1900-01). Mini-prep (alkaline lysis) DNA preparations are adequate for Gateway® 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).
- You may use *att*B-PCR products in the BP reaction without purification. To achieve a higher percentage of desired clones, use PEG/$MgCl_2$ precipitation (see below) to remove primer-dimers or small DNA molecules (< 300 bp).
- To maximize cloning efficiency, 30% PEG 8000/30 mM $MgCl_2$ Solution is provided to purify PCR products away from other DNA < 300 bp in size, including primer-dimers. Simply dilute the PCR reaction 4-fold with TE [10 mM Tris-HCl (pH 7.5-8), 1 mM EDTA], then add 1/2 volume of 30% PEG 8000/30 mM $MgCl_2$ Solution (final concentrations of 10% PEG, 10 mM $MgCl_2$). Vortex to mix thoroughly and centrifuge 15 minutes at full speed in a microcentrifuge. Carefully remove supernatant and suspend the clear pellet in TE to >10 ng/µl. **Important:** Use the recommended proportion of PEG/$MgCl_2$ (4) to ensure that the correct sized products are removed.
- For BP reactions, the most efficient substrates are **linear** *att*B products (PCR products or expression clones) and supercoiled *att*P-containing donor vectors. Supercoiled or relaxed *att*B substrates may be used, but will react less efficiently than linear *att*B substrates.
- To increase the number of colonies containing the desired entry clone, increase the incubation time from the recommended 1 hour to 4-6 hours (typically 2-3 fold more colonies) or overnight (typically 5-10 fold more colonies). Longer incubations are recommended for genes ≥ 5 kb to increase the yield of colonies.
- We recommend using 40-100 fmol of PCR product per 20 µl reaction (where a 1 kb PCR product is ~0.65 ng/fmol). Increasing the amount of PCR product generally yields more colonies; however, do not exceed ~500 ng of PCR product per 20 µl reaction.

Procedures

BP Reaction

Use the following procedure to perform a BP recombination reaction. For a positive control, use 100 ng (2 µl) of pEXP7-tet DNA.

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

*att*B-PCR product (≥ 10 ng/µl; final amt ~30-300 ng)	1-10 µl
Donor vector (150 ng/µl)	2 µl
5X BP Clonase™ Reaction Buffer	4 µl
TE buffer, pH 8.0	to 16 µl

2. Remove BP Clonase™ enzyme mix from -80°C and thaw on ice for about 2 minutes.Vortex the BP Clonase enzyme mix briefly twice (2 seconds each time).
3. To each sample (Step 1, above), add 4 µl of BP Clonase™ enzyme mix to the reaction and mix well by vortexing briefly twice. Microcentrifuge briefly.
4. Return BP Clonase™ enzyme mix to -80°C storage immediately after use.
5. Incubate reactions at 25°C for 60 minutes.
6. Add 2 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 BP reaction into 50 µl of Library Efficiency® DH5α™ 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 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 Library Efficiency® DH5α™ Competent Cells as described above. Plate 20 µl and 100 µl on LB plates containing 100 µg/ml ampicillin.

Expected Results

An efficient BP recombination reaction will produce >1500 colonies if the entire transformation is plated.

Quality Control

BP 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. Jordan, H., and Hartley, J. L. (1996) *Focus® 18*, 26.

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