invitrogen™

Gateway® BP Clonase™ II Enzyme Mix

Cat. No. 11789-020 **Size: 20 reactions**
Cat. No. 11789-100 **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® BP Clonase™ II enzyme mix is a proprietary enzyme and buffer formulation containing the bacteriophage lambda recombination protein Integrase (Int), the *E. coli*-encoded protein Integration Host Factor (IHF) (1), and reaction buffer provided in a single mix for convenient reaction set up. Gateway® BP Clonase™ II enzyme mix catalyzes *in vitro* recombination between an *att*B-PCR product (or *att*B-containing expression clone) and an *att*P-containing donor vector to generate an *att*L-containing entry clone. Store Gateway® BP Clonase™ II enzyme mix at -20°C (non-frost-free freezer) for up to 6 months. For long-term storage, store at -80°C.

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

Quality Control

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

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For technical support, contact tech_service@invitrogen.com.

General Recommendations and Guidelines

- pEXP7-tet is provided for use as a positive control in the BP reaction and contains an approximately 1.4 kb fragment consisting of the tetracycline resistance gene and its promoter flanked by *att*B sites.
- For *att*B-containing expression clones, 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.
- 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).
- 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. Run a 25 µl PCR reaction, dilute the PCR reaction 4-fold with TE [10 mM Tris-HCl (pH 7.5-8), 1 mM EDTA], add 1/2 volume of 30% PEG 8000/30 mM $MgCl_2$ Solution (final concentrations of 10% PEG, 10 mM $MgCl_2$), and vortex. 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/$MgCl_2$ to ensure that 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 20-50 fmol of PCR product per 10 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 ~250 ng of PCR product per 10 µl reaction.

Procedures

BP Reaction

BP Clonase™ II enzyme mix is supplied as a 5X solution. If you wish to scale the reaction volume, make sure the BP Clonase™ II enzyme mix is at a final concentration of 1X. For a positive control, use 100 ng (2 µl) of pEXP7-tet.

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 amount ~15-150 ng)	1-7 µl
Donor vector (150 ng/µl)	1 µl
TE buffer, pH 8.0	to 8 µl

2. Thaw on ice the BP Clonase™ II enzyme mix for about 2 minutes. Vortex the BP Clonase™ II enzyme mix briefly twice (2 seconds each time).
3. To each sample (Step 1, above), add 2 µl of BP Clonase™ II enzyme mix to the reaction and mix well by vortexing briefly twice. Microcentrifuge briefly.
4. Return BP 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 BP 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 BP recombination reaction will produce >1500 colonies if the entire BP 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.

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