

## Technical Data Sheet

**pAChLT-A,B,C Baculovirus Transfer Vector****Product Information**

|                         |                                      |
|-------------------------|--------------------------------------|
| <b>Material Number:</b> | <b>554796</b>                        |
| <b>Component:</b>       | <b>51-21464P</b>                     |
| <b>Description:</b>     | pAChLT-A Baculovirus Transfer Vector |
| <b>Size:</b>            | 20 µg in 20 µl (1 ea)                |
| <b>Component:</b>       | <b>51-21465P</b>                     |
| <b>Description:</b>     | pAChLT-B Baculovirus Transfer Vector |
| <b>Size:</b>            | 20 µg in 20 µl (1 ea)                |
| <b>Component:</b>       | <b>51-21466P</b>                     |
| <b>Description:</b>     | pAChLT-C Baculovirus Transfer Vector |
| <b>Size:</b>            | 20 µg in 20 µl (1 ea)                |

**Description**

The pAChLT-A,B,C baculovirus transfer vectors are derivatives of the pAcG1 vector. They contain a 6xHis tag upstream of the multiple cloning site (MCS) and will express the desired protein as a 6xHis fusion protein. This will substantially ease the purification of the recombinant protein since the 6xHis fusion proteins bind with high affinity to Ni-NTA Agarose. Most host cell proteins do not bind to such a matrix. Therefore, highly efficient **single step affinity purification** can be done with 6xHis-tagged proteins. A protein kinase A site follows the 6xHis motif in the plasmids. This allows the purified proteins to be efficiently phosphorylated with protein kinase A. Usually, this phosphorylation does not alter the binding affinity of the recombinant protein to any of its ligands. If desired, the 6xHis tag can be removed by incubating the fusion protein in presence of **thrombin**. An additional features of these vectors include their expanded multiple cloning site. All inserts **must be in frame with the 6xHis open reading frame (ORF)** in order to be properly expressed. These vectors are recommended for use in conjunction with BD BaculoGold™ baculovirus DNA (Cat. No. 554739) to achieve virtually 100% recombination efficiencies.

**Preparation and Storage**

Store undiluted at -20°C.

The vectors were purified using a silicon bead matrix and dissolved in TE buffer (10 mM Tris-HCl, pH 7.5; 1 mM EDTA).

**Application Notes****Recommended Assay Procedure:**

For expression of a fusion protein under the polyhedrin promoter, insert your gene of interest into a suitable restriction site in frame with the 6xHis ORF in the vector. See attached plasmid maps. Transform and amplify the plasmid DNA in E. coli strains (DH5α, HB101 or any other suitable strain) under ampicillin selection. For construction of recombinant virus, perform a co-transfection of the recombinant pAChLT vector and linearized baculovirus DNA (BaculoGold™ viral DNA Cat. No. 554739) into a susceptible cell line (Sf9 or Sf21). For detailed protocols refer to the Baculovirus Expression Vector System Manual, 6th edition on our web site at <http://www.bdbiosciences.com/pdfs/manuals/98-6088-1F.pdf>.

**Suggested Companion Products**

| <u>Catalog Number</u> | <u>Name</u>                       | <u>Size</u>            | <u>Clone</u> |
|-----------------------|-----------------------------------|------------------------|--------------|
| 554739                | Linearized Baculovirus DNA        | 5 transfections        | (none)       |
| 560129                | Transfection Kit                  | 5 transfections        | (none)       |
| 560138                | 6XHis Purification Kit            | 1 box                  | (none)       |
| 554763                | Sf9 Insect Cells (Live) in TNM-FH | >10 <sup>7</sup> cells | (none)       |
| 554762                | Sf9 Insect Cells (Frozen)         | >10 <sup>7</sup> cells | (none)       |
| 554783                | Thrombin Powder                   | 20 mg                  | (none)       |

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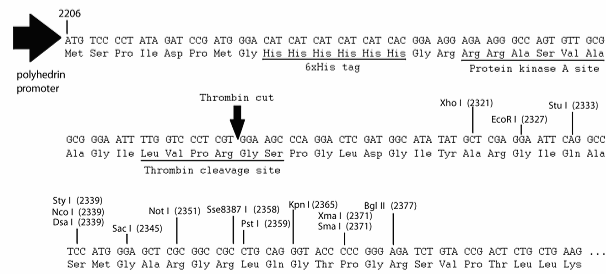
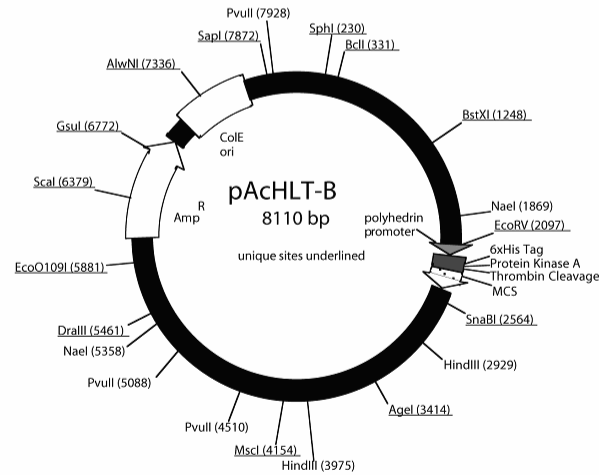
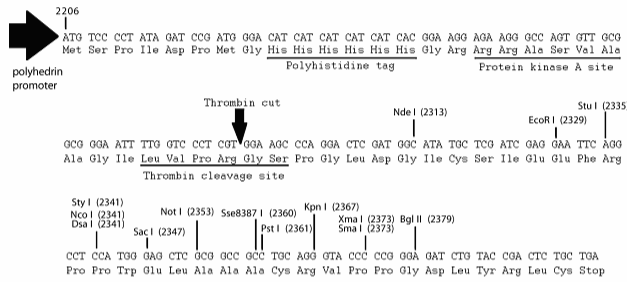
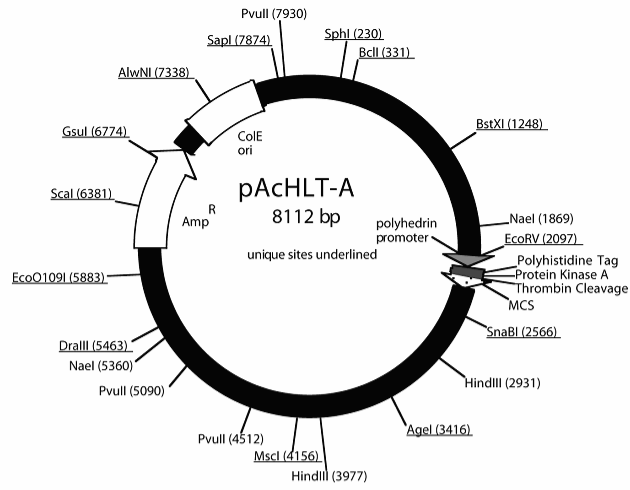
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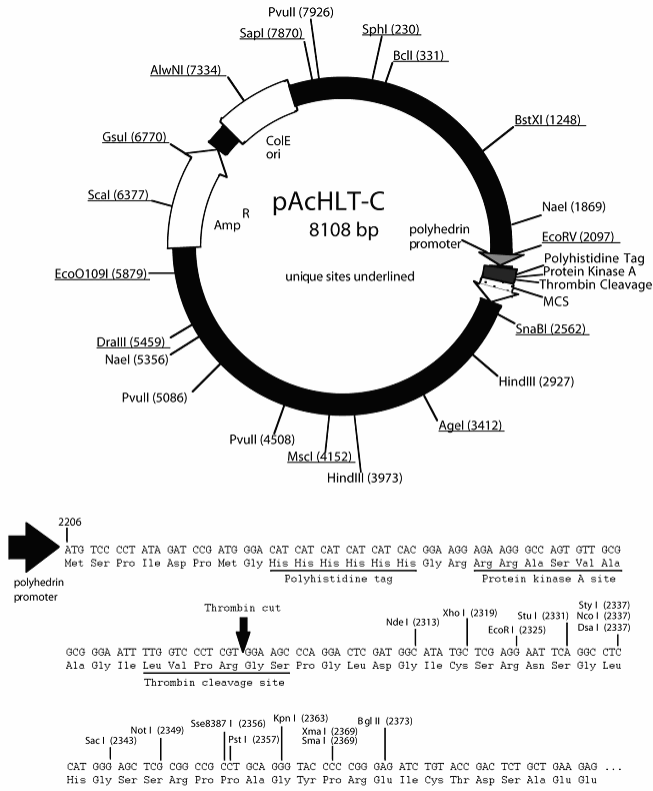
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**Product Notices**

1. Please refer to [www.bdbiosciences.com/pharmingen/protocols](http://www.bdbiosciences.com/pharmingen/protocols) for technical protocols.



**References**

Sambrook J, Fritsch E, Maniatis T. *Molecular Cloning, 2nd Edition*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press; 1989.(Methodology)