**pCLIP Vector**

**pCLIP Vector** is a mammalian expression plasmid intended for the cloning and stable or transient expression of CLIP-tag® protein fusions in mammalian cells. This plasmid encodes CLIP, a CLIP-tag protein, which is expressed under control of the CMV promoter. The expression vector has an IRES (internal ribosome entry site) and a neomycin resistance gene downstream of the CLIP1 for the efficient selection of stable transfectants. pCLIP Vector contains two multiple cloning sites to allow cloning of the fusion partner as a fusion to the N- or C-terminus of the CLIP. The CLIP-tag is a novel tool for protein research, allowing the specific, covalent attachment of virtually any molecule to a protein of interest. The CLIP-tag is a small protein based on human O'-alkylguanine-DNA-alkyltransferase (hAGT). The CLIP-tag substrates are derivatives of benzyl cytosine (BC). In the labeling reaction, the substituted benzyI group of the substrate is covalently attached to the reactive cysteine of CLIP-tag forming a stable thioether link.

pCLIP, contains an improved version of CLIP-tag, termed CLIP, CLIP, displays faster kinetics in 
in vitro labeling and fast, specific and efficient labeling in live and fixed cell applications, thereby rendering it a desired research tool for analysis of protein dynamics.

Although CLIP-tag is based on the same protein as SNAP-tag®, the benzylcytosine substrates form a separate class of substrates, different from the benzylguanine substrates recognized by SNAP-tag. CLIP-tag and SNAP-tag can be used for orthogonal simultaneous labeling.

There are two steps to using this system: subcloning and expression of the protein of interest as a CLIP, fusion, and labeling of the fusion with the CLIP-tag substrate of choice. Cloning and expression of CLIP, fusion proteins are described in this document. The labeling of the fusion proteins with CLIP-tag substrates is described in the instructions supplied with the CLIP-tag substrates.

**Materials Required but not Supplied:**
- Tissue culture reagents and media
- Mammalian cell line(s)
- Transfection reagents

**Storage**
pCLIP, Vector is supplied in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) at a concentration of 0.5 µg/µl. Plasmid solutions can be stored at 4°C for up to one week. For long-term storage, –20°C is recommended.

**Introduction**

**Detailed Description**

A plasmid map and the sequence of the cloning region can be found at the end of these instructions. The complete plasmid sequence can be downloaded at www.neb.com. This plasmid encodes the gene CLIP, which is a mutant form of the human gene for O'-alkylguanine-DNA-alkyltransferase (hAGT). The codon usage of the gene is optimized for expression in mammalian cells. In the plasmid sequence, the CLIP gene is encoded from 969 bp to 1514 bp.

This plasmid is intended for the cloning and stable or transient expression of CLIP-tag protein fusions in mammalian cells. It is suitable for the efficient production of stable cell lines expressing CLIP, gene fusions. The plasmid contains the CMV promoter followed by the genes for CLIP and neomycin resistance separated by the IRES of the encephalomyocarditis virus (ECMV), which permits the translation of two open reading frames from one messenger RNA; therefore after selection of stable mammalian cells for neomycin resistance, nearly all surviving colonies should stably express the CLIP, fusion protein. Unless the expression experiments require a pure population of cells, the pool of resistant cells can simply be used, otherwise cell clones can be isolated and characterized using standard procedures.

The plasmid contains the β-lactamase (Ampicillin resistance) gene for maintenance in bacteria. The gene of interest can be cloned upstream or downstream of the CLIP, coding sequence, as a fusion to the N- or C-terminus of the CLIP-tag.

**Cloning of CLIP-tag Fusions in pCLIP**

**Cloning by PCR**

To subclone the gene of interest into pCLIP, fused to the N-terminus of CLIP, use the available restriction sites: Nhel, EcoRV (blunt), Ascl, SwaI (blunt), BsrGI, AgeI or EcoRI which are located upstream of the CLIP-tag.

To subclone the gene of interest into pCLIP, fused to the C-terminus of CLIP, use the available restriction sites downstream of the CLIP-tag: SbfI, BamHI, Pmel (blunt), XhoI, PacI or NotI.

**Direct Cloning**

Direct cloning can also be used to make fusions with the CLIP-tag. This is only possible if the fusion partner has compatible sites adjacent to the gene of interest.

Care should be taken to design the cloning so that the fusion partners in the resulting construct are in frame.

**Note:** When fusing the gene of interest to the C-terminus of CLIP, note that there is a stop codon between the PacI and NotI sites, so SbfI, BamHI, Pmel, XhoI or PacI must be used as the 5′ cloning site for the insert.

**Note:** Pmel and XhoI cannot be used together for cloning because they share a cysteine as part of their recognition sequences.

**Expression of CLIP-tag Fusions**

**Transient Expression**

Expression of the protein fusion cloned in pCLIP, can be achieved by transiently transfecting cells in culture with standard transfection protocols. The appropriate reagent and time to permit adequate expression must be empirically determined. We recommend using pCLIP-H2B (NEB #N9218) as an expression control plasmid. H2B-CLIP fusion protein gives a nuclear localized signal when labeled with CLIP-Cell substrates. If the empty pCLIP, plasmid is used as a control vector for transfection, an even distribution of the CLIP-tag in nucleus and cytoplasm should be seen. Both pCLIP, and the localization control plasmid have performed well in stable and transient transfection of CHO-K1, COS-7, U-2 OS and NIH 3T3 cells. Note that the intensity of the fluorescence may vary, depending on the cell line and labeling substrate used.

**Stable Expression**

pCLIP, and the localization control plasmids can be transfected by standard transfection methods. Twenty-four to 48 hours after transfection, begin selecting mammalian cultures in 600–1,200 µg/ml G418 (geneticin) depending on the cell line. It is recommended that a kill curve be established for each cell line to determine optimal selection conditions. After 8–12 days of continuous selection, stable colonies will become visible. It is possible to use pools of stable cell populations for initial cell labeling to test for the presence of CLIP-tag expression. In addition, monoclonal cell lines can be isolated and characterized, if desired.
**Troubleshooting**

**Cloning of the Gene of Interest**

If subcloning of the gene of interest with the CLIP-tag does not work, reconfirm all the cloning steps (primer design, choice of restriction sites, DNA isolation, ligation and transformation, etc.). If all steps are confirmed as being correct, then try the cloning using different restriction sites. Be sure to include a positive and negative control for the ligation reaction. Alternatively, try to subclone the CLIP gene into a mammalian expression vector already containing the gene of interest.

**Expression**

In general, we have not experienced problems expressing CLIP-tag protein fusions. However, if the fusion protein does not appear to be expressed, try expressing the H2B-CLIP protein fusion as a positive control using cells transiently transfected with pCLIP-H2B. Labeling of such cells with a fluorescent CLIP-Cell substrate should show strong nuclear localized fluorescence. The empty pCLIP plasmid can also be used as a control (cytosolic and nuclear fluorescence). Counterstaining may help. Signs of host cell toxicity could include the tag does not work, reconfirm all the cloning steps.

**Cloning Region of pCLIP**

Unique restriction sites in the regions flanking the CLIP gene are displayed above the coding strand. The complete sequence for pCLIP and the control plasmids can be downloaded at [www.neb.com](http://www.neb.com).

**Plasmid Map of pCLIP, Vector**

This map and the maps for the control plasmids can be downloaded at [www.neb.com](http://www.neb.com).

**References:**


**Companion Product:**

pCLIP-H2B
#N9218S 20 µg