pCLIP$_f$-Cox8A Control Plasmid

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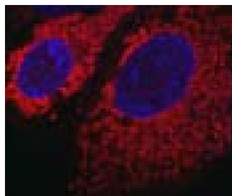
N9217S

20 µg	Lot: 0011012
Store at: –20°C	Exp: 12/12



Live HEK293 cells transiently transfected with pCLIP$_f$-Cox8A. Cells were labeled with CLIP-Cell TMR-Star (red) for 30 minutes and counterstained with Hoechst 33342 (blue).

Introduction

This control plasmid contains the gene encoding the Cytochrome C oxidase, subunit 8-2 (COX8-2) protein cloned upstream of the CLIP$_f$ coding sequence in pCLIP$_f$, as a fusion to the N- terminus of the CLIP-tag. Cytochrome C oxidase is located in the inner mitochondrial membrane and is the terminal enzyme of the respiratory chain. The COX8-2-CLIP$_f$ fusion protein gives mitochondrial fluorescence when labeled with CLIP-Cell™ substrates. The full sequence and map for pCLIP$_f$-Cox8A can be downloaded at www.neb.com.

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 polypeptide based on human O^6-alkylguanine-DNA-alkyltransferase (hAGT). CLIP-tag substrates are derivatives of benzyl cytosine (BC). In the labeling reaction, the substituted benzyl group of the substrate is covalently attached to the reactive cysteine of CLIP-tag forming a stable thioether link. 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.

pCLIP$_f$ contains an improved version of CLIP-tag, termed CLIP$_f$. CLIP$_f$ 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.

There are two steps to using this system: sub-cloning and expression of the protein of interest as a CLIP$_f$ fusion, and labeling of the fusion with the CLIP-tag substrate of choice. Expressing the CLIP$_f$-Cox8A fusion protein is described in this document. The labeling of the fusion proteins with CLIP-tag substrates is described in the instructions supplied with CLIP-tag substrates.

Materials Required but not Supplied:

Cell culture media and reagents
Mammalian cell lines
Transfection reagents
CLIP-tag substrates

Storage

pCLIP$_f$-Cox8A 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.

Expression of CLIP$_f$ Fusions

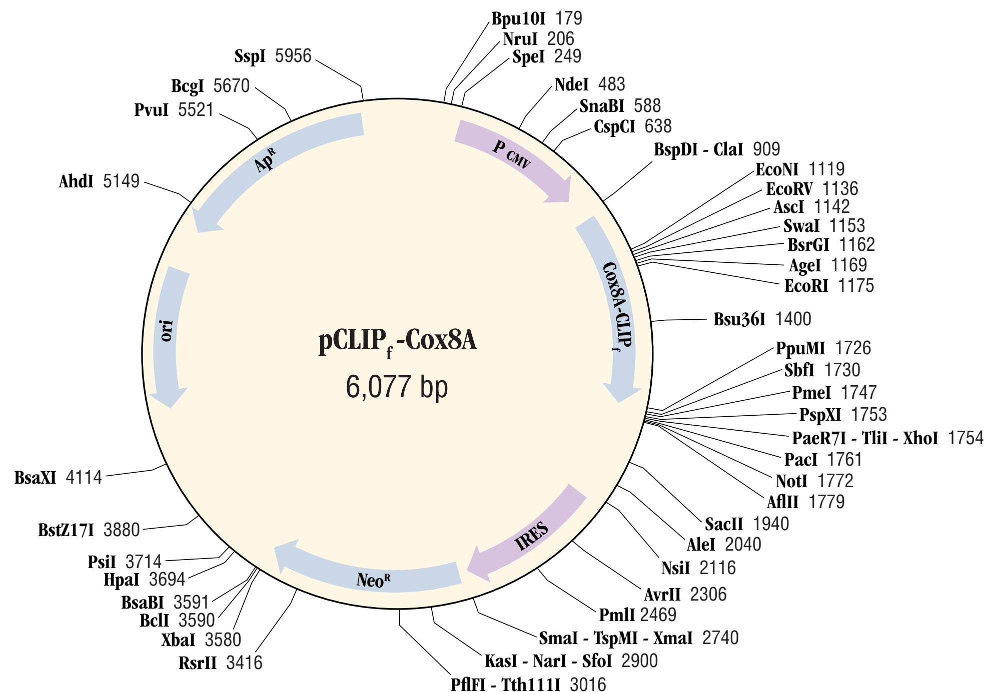
Transient Expression

Expression of the fusion protein cloned in pCLIP$_f$-Cox8A 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. pCLIP$_f$-Cox8A has 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 cell line and labeling substrate used.

We recommend using TransPass D2 (NEB #M2554) in combination with TransPass V (NEB #M2561) or Roche's FuGENE® 6 Transfection Reagent for both transient and stable transfections.

Stable Expression

pCLIP$_f$-Cox8A can be transfected as described above for transient transfection or by other 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 you establish a kill curve 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 clonal cell lines can be isolated and characterized if desired.



Troubleshooting

Expression

In general we have not experienced problems expressing CLIP$_f$-Cox8A from the pCLIP$_f$-Cox8A plasmid. Labeling of transfected cells with a fluorescent CLIP-Cell substrate should show strong mitochondrial fluorescence. In most instances, difficulties in expression can be resolved by altering the transfection protocol.

The CMV promoter is covered under U.S. Patent No. 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

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CERTIFICATE OF ANALYSIS