pCLIP$_f$-NK1R Control Plasmid

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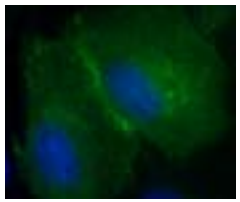
N9216S 001101212120

N9216S

20 µg **Lot: 0011012**

Store at: –20°C **Exp: 12/12**



Live CHO-K1 cells transiently transfected with pCLIP$_f$-NK1R. Cells were labeled with CLIP-Surface™ 488 (green) for 30 minutes and counterstained with Hoechst 33342 (blue).

Introduction

This control plasmid contains the gene encoding the seven-pass transmembrane protein Neurokinin-1 receptor (NK1R), a member of the G-protein coupled receptor family. The NK1R was cloned downstream of the CLIP$_f$ coding sequence in pCLIP$_f$, as a fusion to the C- terminus of the CLIP$_f$.

An import signal sequence for import into the endoplasmic reticulum (ER) was cloned upstream of the CLIP$_f$. This import signal sequence is based on the serotonin receptor 5HT3A. The CLIP$_f$-neurokinin-1 receptor is inserted in the plasma membrane with the CLIP$_f$ exposed to the extracellular side of the membrane. The CLIP$_f$-NK1R fusion protein gives plasma membrane labeling when labeled with cell permeable CLIP-Cell™ substrates and non-cell permeable CLIP-Surface substrates.

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 (AGT). CLIP-tag substrates are derivatives of benzylcytosine (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: subcloning and expression of the protein of interest as a CLIP$_f$ fusion, and labeling of the fusion with the CLIP-tag substrate of choice. Expression of CLIP$_f$-NKIR 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 reagents and media

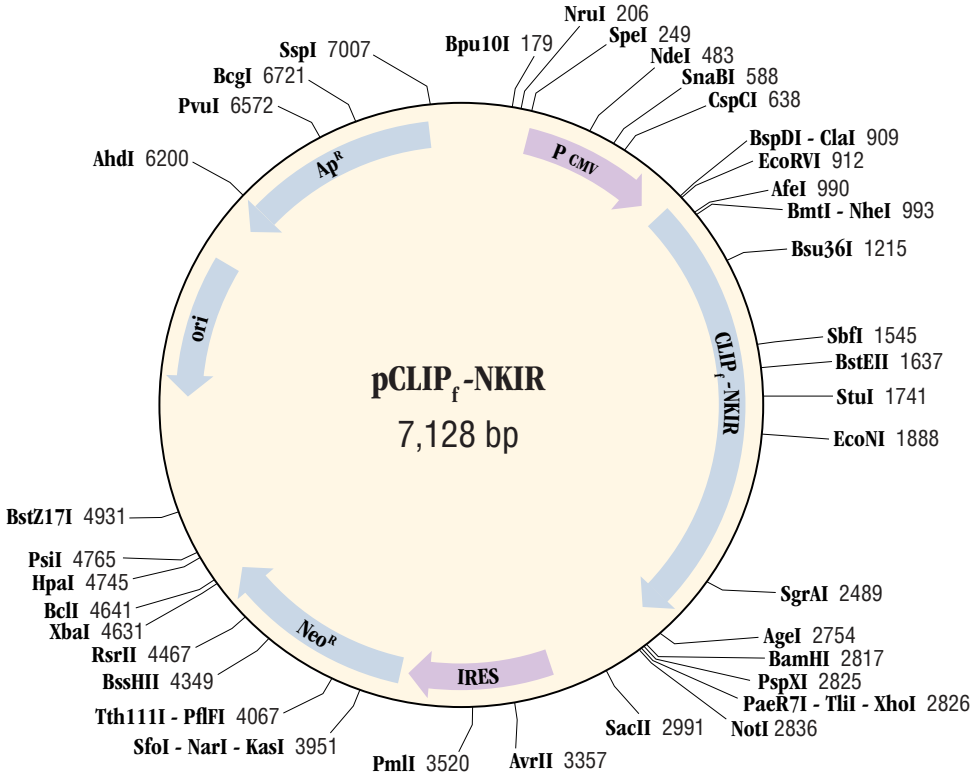
Mammalian cell lines

Transfection reagents

CLIP-tag substrates

Storage

pCLIP$_f$-NK1R 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$_f$-NK1R 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$_f$-NK1R 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$_f$-NK1R 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 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 clonal cell lines can be isolated and characterized if desired.

Troubleshooting

Expression

In general, we have not experienced problems expressing CLIP$_f$-NK1R from the pCLIP$_f$-NK1R plasmid. Labeling of transfected cells with a fluorescent CLIP-Cell or CLIP-Surface substrate should show strong cell surface fluorescence. In most instances, difficulties in expression can be resolved by altering the transfection protocol.

CERTIFICATE OF ANALYSIS