

# CLIP-Cell™ Block



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59220S 004140317031

## S9220S

**100 nmol** Lot: **0041403**  
**Store at -20C** Exp: **3/17**

### Introduction

CLIP-Cell™ Block (bromothienylcytosine, BTC) is a non-fluorescent compound that blocks the reactivity of the CLIP-tag™ in solution and inside or on the surface of living cells. It can be used to generate inactive controls in live cell labeling experiments performed with CLIP-tag fusion proteins. CLIP-Cell Block reacts with CLIP-tag irreversibly, inactivating it for subsequent labeling steps.

The CLIP-tag is a novel tool for protein research, allowing the specific, covalent attachment of virtually any molecule to a protein of interest. CLIP-tag is a small polypeptide based on mammalian O<sup>6</sup>-alkylguanine-DNA-alkyltransferase (AGT). CLIP-tag substrates are derivatives of benzylcytosine. 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.

There are two steps to using this system: sub-cloning and expression of the protein of interest as a CLIP-tag fusion, and labeling of the fusion with the CLIP-tag substrate of choice. The use of CLIP-Cell Block during the labeling of fusion proteins with CLIP-Cell substrates is described below.

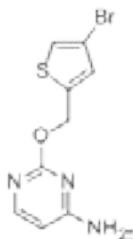


Figure 1. Structure of CLIP-Cell Block (MW 286.1 g/mol)

### Materials required but not supplied:

Cells expressing CLIP-tag fusion proteins  
Tissue culture materials and media  
Transfection reagents  
Fluorescence microscope with suitable filter set  
DMSO

### Storage

CLIP-Cell Block should be stored at -20°C (long term) or at 4°C (short term). With proper storage at -20°C, CLIP-Cell Block is stable for at least two years dry or for three months when dissolved in DMSO.

### Quality Controls

**Purity and Characterization:** Purity of CLIP-Cell Block was determined to be 97% by HPLC analysis. Molecular weight [M+H]<sup>+</sup> was determined by MS to be 285.9 (286.0 expected).

**In vitro Protein Labeling:** Reaction of CLIP-Cell Block (10 µM) with purified CLIP-tag protein (5 µM) *in vitro*, followed by mass spec analysis, indicated an efficiency of labeling of ≥ 95%.

**Blocking of Cellular Protein Labeling:** Cells transfected with CLIP-tag vector expressing CLIP-H2B (intracellular) were reacted sequentially with 10 µM CLIP-Cell Block for 30 minutes, then 3 µM CLIP-Cell TMR-Star for 60 minutes. Cells transfected with CLIP-tag vector expressing CLIP-NK1R (surface) were reacted sequentially with 10 µM CLIP-Cell Block for 30 minutes, then 5 µM CLIP-Surface™ 547 for 60 minutes. No labeling was detected in the blocked cells by confocal microscopy.

### Instructions for Use with CLIP-tag Substrates

In many cases the labeling of a non-transfected cell sample or a mock-transfected cell sample will be completely sufficient as a negative control for cell labeling. In some cases, however, it may be desirable to selectively block the CLIP-tag activity in a cell sample expressing the CLIP-tag fusion protein to generate a control. This is done by a pre-incubation of the cells with CLIP-Cell Block, followed by the incubation with the labeling solution. CLIP-Cell Block may also be used in pulse-chase experiments to block the CLIP-tag reactivity during the chase between two pulse-labeling steps.

**Note:** under the conditions given below, CLIP-Cell Block blocks most of the active CLIP-tag. However, achievement of complete blocking may be dependent on experimental conditions. Always take care to avoid carryover of CLIP-Cell Block to samples that you do not wish to block.

The following steps describe the use of CLIP-Cell Block in a typical labeling experiment:

1. Dissolve one tube of CLIP-Cell Block (100 nmol) by adding 50 µl of DMSO to give a solution of 2 mM CLIP-Cell Block. Mix by vortexing for 10 minutes, until all the CLIP-Cell Block is dissolved. Store this stock solution in the dark at 4°C or for extended storage at -20°C. We recommend using a final concentration of 10 µM, which is a 1:200 dilution of this stock solution.
2. Prepare two cell samples suitable for labeling, expressing the CLIP-tag fusion protein of interest.
3. Mix an appropriate amount of medium with CLIP-Cell Block stock solution in a ratio of 1:200 to give a blocking medium of 10 µM CLIP-Cell Block. For best performance, add the dissolved CLIP-Cell Block to complete medium, including serum. Do not prepare more medium with CLIP-Cell Block than you will consume within one hour.
4. Mix an appropriate amount of medium with DMSO in a ratio of 1:200, to give a final concentration of 0.5% v/v DMSO.
5. Replace the medium on one sample of cells with the blocking medium. These are your Blocked Cells. Replace the medium on the other sample of cells with the medium containing DMSO. These are your Test Cells. Incubate both cell samples for 30 minutes.

Number of Wells in Plate	Recommended Volume for Cell Labeling
6	1 ml
12	500 µl
24	250 µl
48	100 µl
96	50 µl

These recommendations are for culturing cells in polystyrene plates. For confocal imaging, we recommend using chambered coverglass such as Lab-Tek II Chambered Coverglass which is available in a 1, 2, 4 or 8 well format from Nunc ([www.nuncbrand.com](http://www.nuncbrand.com)).

6. Remove CLIP-Cell Block or DMSO containing medium by washing both samples of cells twice with complete medium.
7. Label both cell samples with the fluorescent CLIP-Cell substrate using the supplied protocol.
8. Inspect both samples under the fluorescence microscope. The Blocked Cells should show no fluorescence, whereas the Test Cells should show fluorescence localized to where the CLIP-tag fusion protein is present in the cell.

### Note

Please note that there is a constant turnover and resynthesis of proteins in the cell. After having blocked all existing CLIP-tag fusion proteins within the cell, new CLIP-tag fusion protein molecules may be synthesized in the meantime and may get labeled during incubation with a fluorescent CLIP-tag substrate. This will give the impression that the blocking was ineffective. In order to minimize these effects of protein synthesis and protein transport, cells may have to be treated with cycloheximide and incubation with the fluorescent CLIP-tag substrate may have to be performed at 4°C.

### Instructions for Labeling of Proteins *in vitro*:

1. Dissolve the vial of CLIP-Cell Block (100 nmol) in 50 µl of fresh DMSO to yield a labeling stock solution of 2 mM CLIP-Cell Block. Mix by vortexing for 10 minutes until all the CLIP-tag substrate is dissolved. Dilute this 2 mM stock solution 1:4 in fresh DMSO to yield a 500 µM stock for labeling proteins *in vitro*.
2. Set up the reactions, in order, as follows:

Component	Volume	Final Concentration
Deionized Water	30 µl	
5X CLIP-tag Reaction Buffer	10 µl	1X
50 mM DTT	1 µl	1 mM
50 µM CLIP-tag Purified Protein	5 µl	5 µM
500 µM CLIP-Cell Block	2 µl	20 µM
250 µM CLIP-tag Substrate	2 µl	10 µM
<b>Total Volume</b>	<b>50 µl</b>	

3. Incubate sample containing only 20  $\mu\text{M}$  CLIP-Cell Block in the dark for 20 minutes at 37°C.
4. Once incubation with CLIP-Cell Block is complete, add 2  $\mu\text{l}$  of 250  $\mu\text{M}$  CLIP-tag substrate, mix and incubate in the dark for 60 minutes at 37°C.
5. Run sample on an SDS-PAGE gel and detect using a fluorescent gel scanner or store samples at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$  in the dark.

#### **Removal of Unreacted Substrate (optional)**

After the labeling reaction the unreacted substrate can be separated from the labeled CLIP-tag fusion protein by gel filtration or dialysis. Please refer to the vendor's instructions for the separation tools you are using.

#### **Notes for Labeling *in vitro***

We recommend the routine addition of 1 mM DTT to all buffers used for handling, labeling and storage of the CLIP-tag. The stability of the CLIP-tag is improved in the presence of reducing agents; however it can also be labeled in their absence, if handling at temperatures above 4°C is minimized.

CLIP-tag fusion proteins can be purified before labeling, but the labeling reaction also works in non-purified protein solutions (including cell lysates).

#### **Troubleshooting**

For troubleshooting please refer to the instructions supplied with CLIP-Cell products as appropriate.

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