CLIP-Vista Green

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S9235S 0021210151O1

S9235S

50 nmol **Lot: 0021210**

Store at: –20°C **Exp: 10/15**

Introduction

CLIP-Vista Green is a green fluorescent substrate that can be used to label CLIP-tag™ fusion proteins (in cell lysates or purified proteins) for detection by SDS-PAGE. This substrate (BC-Vista Green) is based on fluorescein and is optimized for excitation with the 488 nm laser excitation line in a laser based gel scanner. It can also be excited using 360 nm light from a standard UV-transilluminator. It has an excitation maximum at 500 nm and emission maxima at 524 nm. This package includes 50 nmol of CLIP-Vista Green substrate, sufficient to label one hundred 20 µl samples containing CLIP-tag fusion protein for in-gel detection.

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 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.

There are two steps to using this system: subcloning and expression of the protein of interest as a CLIP-tag fusion, and labeling of the fusion with the CLIP-tag substrate of choice. Expression of CLIP-tag fusion proteins is described in the documentation supplied with CLIP-tag plasmids. The labeling of the fusion proteins with the CLIP-tag substrate for detection by SDS-PAGE is described in this document.

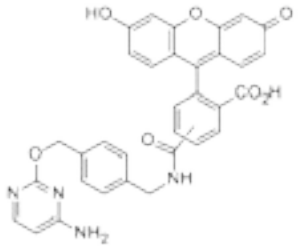

Figure 1. *Structure of CLIP-Vista Green (MW 588.6 g/mol)*

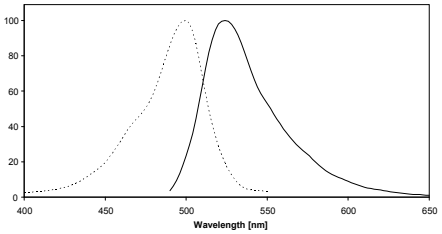

Figure 2. *Excitation (dotted line) and emission spectra of CLIP-Vista Green coupled to CLIP-tag in buffer at pH 7.5*

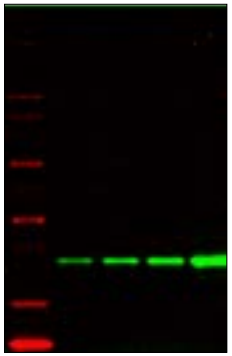

Figure 3. *Typical SDS-PAGE of CLIP-Vista Green labeled proteins visualized using a gel scanner. The gel was imaged with a Typhoon 9400 imager at 300V PMT with the 488/526 nm excitation/ emission filter set. Lane 1 fluorescent MW markers (Fermentas; SM0671); Lanes 2–5 CLIP-His (22 kDa) 50, 150, 300, and 600 ng.*

Storage

Store substrate at –20°C. After dissolving CLIP-Vista Green store it in aliquots at –20°C in the dark for up to three months. Once an aliquot is thawed it can be stored at 4°C in the dark for up to two weeks. Make sure substrate, which may precipitate during freezing, is completely dissolved before use.

To make a stock solution, dissolve substrate in 200 µl of a general purpose protein buffer containing 1 mM DTT (e.g., 50 mM Tris, 100 mM NaCl, 0.1% Tween 20, 1 mM DTT, pH 7.5–8.0). Mix by vortexing for 10 minutes or until all substrate is dissolved. Dispense into 50 µl aliquots for storage.

Quality Controls

Purity and Characterization: Purity of CLIP-Vista Green was determined to be 89% by HPLC analysis. Molecular weight $[M+H]^+$ was determined by MS to be 589.2 (589.2 expected).

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

Instructions for Use

Protocol for Labeling Mammalian Cell Lysates

1. Harvest cells by trypsinization following established protocols.
2. Wash cells twice with PBS.
3. Lyse cells by suspending in reaction buffer at 10^4–10^5 cells per 20 µl. Reaction buffer is 50 mM Tris–HCl, pH 7.5, 100 mM NaCl, 0.1% Tween 20, 1 mM DTT and an EDTA-free protease inhibitor cocktail (e.g., Complete™, Roche).
4. Add 2 µl of the CLIP-Vista Green stock solution to 18 µl of cell lysate. Mix well by pipetting up and down several times.
5. Incubate in the dark for 60 minutes at 37°C.
6. Add an appropriate volume of concentrated SDS-PAGE sample buffer and proceed with sample preparation and SDS-PAGE according to the gel manufacturer's instructions.
7. After the gel is run, immediately obtain a fluorescent image using a laser scanner with 488 nm excitation or a UV transilluminator and an appropriate camera (Polaroid or digital). Excitation at 488 nm will give the best results. The fluorescence is an intense green.
8. After fluorescent imaging, standard fixing and staining protocols can be used to detect the non-fluorescent proteins.

Protocol for Labeling Purified Proteins *in vitro*:

1. Add 2 µl of the substrate stock solution to 18 µl of protein sample containing a CLIP-tag fusion protein in an appropriate buffer (see notes). Mix well by pipetting up and down several times.
2. Incubate in the dark for 60 minutes at 37°C.
3. Add an appropriate volume of concentrated SDS-PAGE sample buffer and proceed with sample preparation and SDS-PAGE according to the gel manufacturer's instructions.
4. After the gel is run, immediately take a fluorescent image using a laser scanner with 488 nm excitation or a UV-transilluminator and an appropriate camera (Polaroid or digital). Excitation at 488 nm will give the best results. The fluorescence is an intense green.
5. After fluorescent imaging, standard fixing and staining protocols can be used to detect the non-fluorescent proteins.

Notes

CLIP-Vista Green is dried down with mannitol to improve its aqueous solubility. Mannitol will be present at 10 mM final concentration and should not lead to any problems.

Most gel fixing/staining protocols will affect the fluorescence of the CLIP-Vista Green substrate. The fluorescent gel image should be appropriately documented before continuing with protein staining.

We recommend the routine addition of 1 mM DTT to all buffers used for handling, labeling and storage of the CLIP-tag. This will enhance the labeling by improving the stability and reactivity of the CLIP-tag fusion protein. Labeling also works under non-reducing conditions. Care should be taken to avoid handling the CLIP-tag fusion protein above 4°C prior to labeling.

Where stickiness of the fusion protein is a problem we recommend adding Tween 20 at a final concentration of 0.05% to 0.1%. At this concentration Tween 20 does not affect the performance of the CLIP-tag.

Correct storage and handling of unlabeled CLIP-tag fusion proteins is essential to maintain reactivity of the CLIP-tag prior to labeling. Unlabeled fusion proteins should be stored at –20°C and thawed just before use. Prolonged handling at temperatures above 4°C should be avoided, especially if the protein is stored in the absence of reducing agents (e.g., DTT).

The CLIP-tag labeling reaction is tolerant of a wide range of buffers. The requirements of the fusion partner should dictate the buffer selected. The following buffer guidelines are recommended: pH between 7.0 and 8.0, monovalent salts (e.g. sodium chloride) between 50 mM and 250 mM, at least 1 mM DTT. Non-ionic detergents can

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be added to 0.5% v/v if required, but SDS and other ionic detergents should be avoided entirely because they inhibit the activity of the CLIP-tag. Metal chelating reagents (e.g., EDTA and EGTA) also inhibit CLIP-tag activity and should be avoided. Many proteins benefit from the addition of glycerol for frozen storage, typically 20% v/v.

Unreacted CLIP-Vista Green substrate will run in front of the protein bands in the gel, running at an equivalent molecular weight below 10 kDa (below the band obtained for CLIP-tag alone). If the fluorescence from the unreacted substrate interferes with imaging for your protein, you may separate the labeled protein from unreacted substrate after the labeling reaction and before running the gel using, for example, a spin separation device.

Troubleshooting

Labeling Reaction

If solubility problems occur, we recommend testing a range of pH (pH 7.0–pH 8.0). The salt concentration (50–250 mM) may also need to be optimized for your particular fusion protein.

If stickiness of the fusion protein is a problem we recommend adding Tween 20 at a final concentration of 0.05% to 0.1%. The CLIP-tag activity is not affected by this concentration of Tween 20.