FLASH Reagent

Catalog number: 22330
Unit size: 100 Tests

Component	Storage	Amount
FIASH Reagent	Freeze (< -15 °C), Minimize light exposure	100 Tests

OVERVIEW

FLASH is a fluorescein derivative, modified to contain two arsenic atoms at a set distance from each other. It was developed by Roger Tsien and colleagues in 1998. The biarsenical labeling technology works through the high-affinity interaction of arsenic for thiols. When FlASH binds to tetracysteine (TC) sequences, its biarsenical group reacts rapidly with Cys-Cys moiety and the tag becomes highly fluorescent in green. The biarsenical labeling reagent FLASH is the smallest expression tag for labeling a protein that contains a six-amino acid motif with a Cys-Cys-X1-X2-Cys-Cys amino acid sequence. The most commonly used tetracysteine is the six amino acid Cys-Cys-Pro-Gly-Cys-Cys sequence. As this sequence rarely appears in endogenous proteins, incorporating the sequence into target proteins generates a small but highly specific target for protein labeling. FLASH generates a strong green fluorescent signal when binding to recombinant proteins containing the tetracysteine motif Cys-Cys-Pro-Gly-Cys-Cys. It can be used for monitoring protein localization, turnover and trafficking, receptor signaling and internalization.

AT A GLANCE

Protocol summary

1. Prepare cells
2. Prepare FLASH Reagent working solution
3. Incubate the cells with FLASH Reagent working solution for 15-60 minutes
4. Image the cells using the FITC filter set

KEY PARAMETERS

Fluorescence microscope

Excitation	FITC filter set
Emission	FITC filter set
Recommended plate	Black wall/clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

FLASH Reagent stock solution (800X)
Add 67 µL of DMSO to the vial and mix well.

Note Make single used aliquots and store at - 20 °C. Avoid freeze and thaw cycle.

PREPARATION OF WORKING SOLUTION

FLASH Reagent working solution (1X)
Dilute the 800X stock solution at 1:800 in an appropriate buffer such as serum-free or low-level serum (~1%) medium, HHBS, or Opti-MEM® medium to make a 1X labeling solution and mix well.

Note Make the working solution just before use.

Note For cells transduced with lentivirus, a 0.5X working solution may be optimal. Depending on the levels of specific and background fluorescent signal, you can optimize the working solution to better visualize your labeled protein. We recommend trying a concentration range of 0.4 to 4X working solution.
Table 1. The following table is the suggested volume of labeling solution to use for different tissue culture formats.

Plate	96-well	48-well	24-well	12-well	6-well
Volume	100 µL	150 µL	250 µL	500 µL	1 mL

SAMPLE EXPERIMENTAL PROTOCOL

1. Prepare cells as desired.
2. Remove the growth medium from the cells and wash cells once with an appropriate medium.
3. Add the appropriate amount of 1X FLASH Reagent working solution to each well (See table for the appropriate volume).

   Note Appropriately discard any unused 1X working solution according to your institution's guidelines. Do not reuse the 1X working solution.
4. Incubate the cells at room temperature for 15-60 minutes, protected from light.
5. Wash the cells with 250 µM BAL in serum free medium or buffer of your choice 2 times.
6. Image your labeled protein using a fluorescence microscope with a FITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

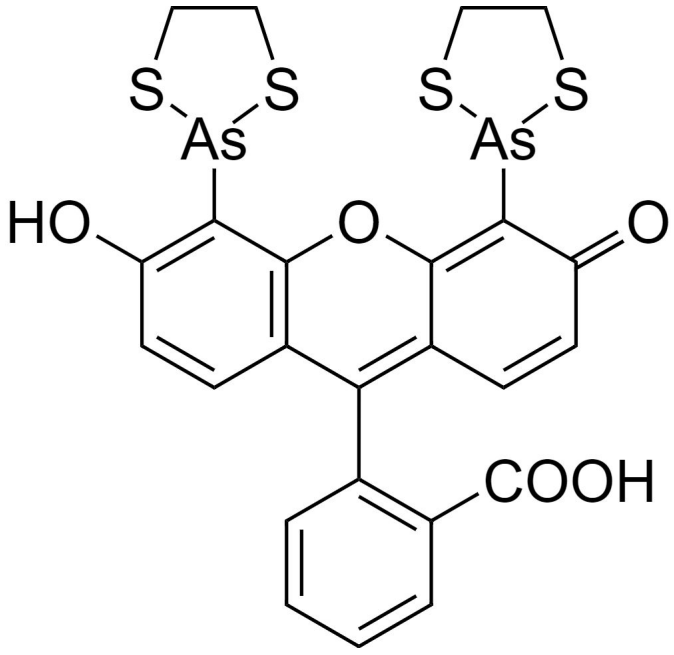

Figure 1. Chemical structure for FIASH Reagent.

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