Tetracysteine REASH Reagent *2 mM DMSO Solution*

Catalog number: 22332
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 22332)
REASH Reagent	Freeze (< -15 °C), Minimize light exposure	100 Tests

OVERVIEW

Tetracysteine REASH Reagent is a resorufin-derived biarsenical probe modified with two arsenic atoms positioned to interact with tetracysteine-containing protein tags. The biarsenical labeling technology is based on the high-affinity interaction between arsenic groups and thiols. Tetracysteine REASH Reagent binds recombinant proteins engineered with a tetracysteine sequence, typically a Cys-Cys-X1-X2-Cys-Cys motif. When REASH binds to the tetracysteine sequence, its biarsenical group reacts rapidly with the Cys-Cys moiety, generating a strong red fluorescent signal. This compact tag-based labeling approach is useful for studying protein localization, turnover, trafficking, receptor signaling, and internalization.

AT A GLANCE

Protocol summary

1. Prepare cells
2. Prepare REASH Reagent working solution
3. Incubate the cells with REASH Reagent working solution for 15-60 minutes
4. Image the cells using the Cy3 filter set

KEY PARAMETERS

Fluorescence microscope

Excitation	Cy3 filter set
Emission	Cy3 filter set
Recommended plate	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

REASH Reagent working solution (1X)

Dilute the 2mM 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 2.5 µM (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 REASH Reagent working solution to each well (See table for the appropriate volume).

   Note: Discard any unused 1X working solution. 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 Cy3 filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

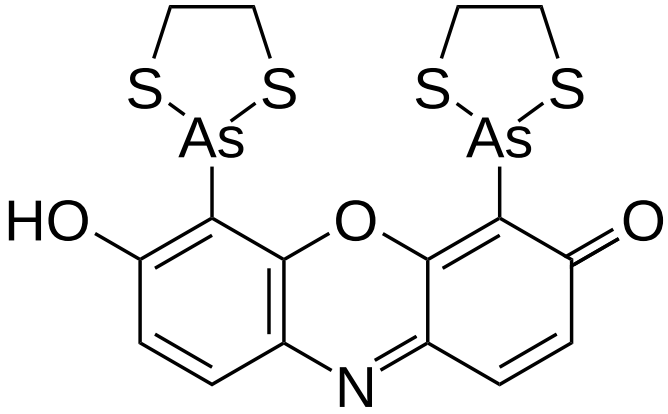

Figure 1. Chemical structure for REASH Reagent.

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