

ab113853 – CFSE Fluorescent Cell Labeling Kit

For the durable fluorescent labeling of live cells for fluorescent microscopy and flow cytometry, population growth studies and within sample duplexing

[View kit datasheet: www.abcam.com/ab113853](http://www.abcam.com/ab113853)

(use www.abcam.cn/ab113853 for China, or www.abcam.co.jp/ab113853 for Japan)

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

CFSE (5(6)-Carboxyfluorescein diacetate N-hydroxysuccinimidyl ester) is a cell permeant, non-fluorescent pro-dye. Intracellular esterases in live cells cleave the acetate groups which results in the green fluorescent molecule carboxyfluorescein that is now membrane impermeant. The succinimidyl ester group reacts indiscriminately with intracellular free amines to generate covalent dye-protein conjugates. The result is live cells with an intracellular fluorescent label. At appropriate concentrations CFSE is non-toxic to cells and the fluorescence is retained after formaldehyde and alcohol fixation. CFSE labeled cells can be detected with any instrument or filter set compatible with fluorescein detection: Excitation(max)=492nm, Emission(max)=517nm.

CFSE is a versatile tool for the fluorescent intracellular labeling of live cells. Labeled cells can be assayed using flow cytometry and fluorescent microscopy. The dye is long lasting and well retained within labeled cells. The provided CFSE is sufficient for ~1000 assays.

CFSE is a cell permeant green fluorescent molecule. The succinimidyl ester group reacts indiscriminately with intracellular free amines to generate covalent dye-protein conjugates. The result is live cells with an intracellular fluorescent label. At appropriate concentrations CFSE is non-toxic to cells and the fluorescence is retained after formaldehyde and alcohol fixation. CFSE labeled cells can be detected with any instrument or filter set compatible with FITC detection: Excitation(max)=492nm, Emission(max)=517nm.

This product document provides a protocol for labeling cells with CFSE and sample applications in which CFSE can be used. Sample experiments include:

1. To label cells with CFSE for use in microscopy as a general stain for cell morphology or as a counterstain for a red fluorophore.
2. To quantify cell proliferation in vitro based on dilution of the dye as parental labeled cells divide.
3. To allow the within sample duplexing by mixing differentially treated CFSE labeled and unlabeled cells in the same sample and subjecting to individual cell analysis (e.g. flow cytometry).

Limitations:

- FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC PROCEDURES.
- Use this kit before expiration date.
- Do not mix or substitute reagents from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

2. Assay Summary

Label live cells with CFSE: Dilute CFSE in PBS or cell culture media

Cells can be in suspension or growing as an adherent monolayer



Incubate 10 minutes at 37°C or room temperature



Wash cells with media to remove non-incorporated dye



Culture and treat labeled cells using standard methods

Harvest cells at desired timepoint



CFSE can be detected by flow cytometry or fluorescence microscopy

CFSE can be detected in live or fixed cells
(Emission(max): 495nm, Excitation(max): 517nm)

3. Kit Contents

- 10mM CFSE in DMSO (1000x): 0.1 mL

4. Storage and Handling

Store CFSE at 4°C if using within one month of receipt. For longer term storage, aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles and protect from light. Allow the product to warm to room temperature before opening. Promptly use diluted solutions of CFSE.

5. Additional Materials Required

- Flow cytometer and/or fluorescence microscope (required excitation/emission wavelengths 495nm/517nm)
- General tissue culture supplies
- PBS (sterile)

6. Assay Procedure

1. Label live cells with 10nM—10 μ M of CFSE

- a. For uniform labeling, cells are best labeled in as a single-cell suspension. Detach cells, by for example trypsin which should then be quenched with media. Cells that grow in suspension should be gently pipetted to dissociate any aggregated cells. It is also possible to label cells growing as a monolayer by first removing the existing media and then overlaying the CFSE staining solution.
 - b. CFSE can be diluted in PBS or culture media. Labeling efficiency will be higher if CFSE is diluted in PBS. If labeling in media, greater concentrations of CFSE are required.
 - c. CFSE should be quickly mixed with cells for most uniform labeling. For example, cells could be fully resuspended in one volume of PBS and then an additional volume of 2X CFSE concentration is added and mixed. Alternatively, pelleted cells could be resuspended in a 1X CFSE staining solution.
 - d. Concentrations of CFSE staining will need to be determined individually based on the cell line used and the goals of the experiment. For example, if following cell proliferation by CFSE dilution, higher levels of CFSE staining is desired. If using CFSE to label cells as a counterstain for additional fluorescent markers, lower levels of CFSE staining would prevent fluorescent spillover between filter sets.
2. Incubate cells in CFSE staining solution for 10-15 minutes at room temperature or at 37°C.

- 3. Quench staining and wash cells with culture media to remove unincorporated CFSE.**
 - a. Add equal volume of culture media (containing serum) to the cells + CFSE staining solution and allow to sit for 5 minutes.
 - b. Next, remove the CFSE-containing solution and wash cells one time with an equal volume of culture media.
 - c. Cells are now fluorescently labeled.
- 4. Return cells to standard culture conditions or immediately assay.**

IMPORTANT ADDITIONAL NOTES:

1. CFSE is amine group reactive. Do not use buffers that contain primary amines during the CFSE labeling step. PBS is the recommended CFSE dilution buffer.
2. The amount of cellular fluorescence drops substantially in the first 24 hours after CFSE labeling. After that, the fluorescence is stably incorporated and will decrease over time as a function of dilution with cell division. If optimizing CFSE labeling concentrations for long-term studies, it is best to optimize to measurements taken at 24 hours post CFSE labeling.

7. Sample Data

i. Fluorescent cell labeling for microscopy:

CFSE is useful as a tool to label live cells. Since the fluorescence is dependent on esterase activity, only live cells are labeled. Since the entire cell is labeled, CFSE can be a useful counterstain when using red fluorophore markers of specific subcellular structures. CFSE has also been used to label cells that are subsequently injected into animals; in this manner implanted cells' migration, morphology and division can be monitored in-vivo.

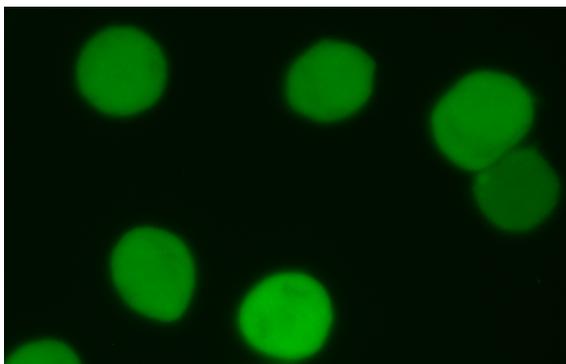


Figure 1. Jurkat cells labeled with CFSE. Jurkat cells were labeled with 1mM CFSE in media for 15 minutes, washed once with PBS and imaged on a fluorescence microscope. The cells in this image are live but fixed cells give similar results.

ii. Tracking cell division with CFSE:

Labeling cells with CFSE can be very useful for tracking cell division in in-vitro cell cultures. As a labeled cell divides, the CFSE fluorescence is partitioned between the daughter cells. Hence, by tracking mean CFSE

intensity per cell over time, one can quantitate cell proliferation.

As an example experiment, Jurkat cells were labeled with CFSE on Day 0 and then monitored at 24 hour intervals for 7 days for CFSE fluorescence by flow cytometry. Figure 2 shows sample flow cytometry histograms and Figure 3 shows a graphical analysis. The thymidine cell cycle arrest in Figure 2C demonstrates that CFSE dilution is dependent on cell division.

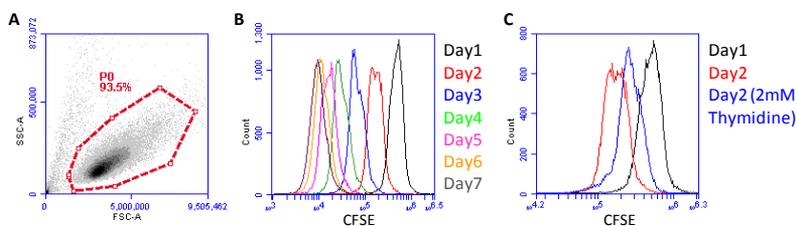


Figure 2. Flow cytometry analysis of CFSE dilution with cell division. Jurkat cells were labeled with $1\mu\text{M}$ CFSE on day0 and then a portion of the culture was subjected to flow cytometry analysis on days 1-7. (A) Example of live cell gating used in this analysis. (B) Overlay histogram of the daily CFSE fluorescence intensity. Mean CFSE intensity per cell decreases as the cells divide and the dye is diluted amongst the daughter cells. (C) As a control, 2mM Thymidine was added to a parallel culture of the day 0 CFSE labeled cells at day1. 2mM thymidine causes an S-phase cell cycle arrest. At day2, there is less dilution of CFSE in the thymidine culture (blue) relative to the untreated culture (red) due to decreased cell division.

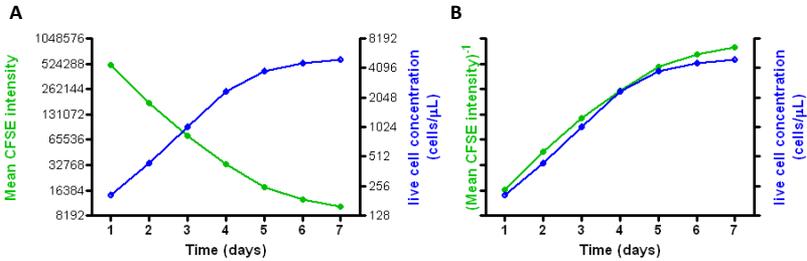


Figure 3. Comparing CFSE dilution with cell count: 7 day experiment.

Graphical analysis of flow cytometry CFSE dilution data from Figure 1 and comparison to cell concentration data. (A) Plot of CFSE fluorescent intensity (green) and cell concentration (blue) as a function of time; each y-axis axis is a log₂ scale. Note that the rate of decrease in CFSE intensity slows and the rate of increase in cell concentration slows as the cell density increases. (B) Re-plot of the data in (A) such that the inverse of CFSE intensity increase is plotted. Note the excellent agreement between CFSE dilution and cell concentration.

Note that the use of CFSE allows the specific tracking of labeled cells in mixed cell populations. For example, proliferation of CFSE-labeled cells could be monitored when co-cultured with other non-labeled cell lines. This could be particularly useful in quantitative evaluation of co-culture systems for isolated primary cells.

iii. Experimental duplexing using CFSE:

Immunostaining a mixture of CFSE-labeled and unlabeled cells allows within-sample duplexing of treated and untreated cells when single cell analysis is available (e.g. flow cytometry, high content microscopy). In this way, treated and untreated cells can be analyzed simultaneously. This doubles throughput while simultaneously providing an excellently controlled experimental setup by eliminating potential for differences in reagent addition between control and experimental samples.

To demonstrate this technique, CFSE labeling was combined with deferoxamine (DFO) treatments. DFO is a hypoxia mimetic and causes the stabilization of the hypoxia induced transcription factor HIF1A. Figure 4 demonstrates control of the cell population by manipulating CFSE labeling and DFO exposure. Figure 5 shows an example experiment with differential immunostaining between CFSE labeled and unlabeled cells.

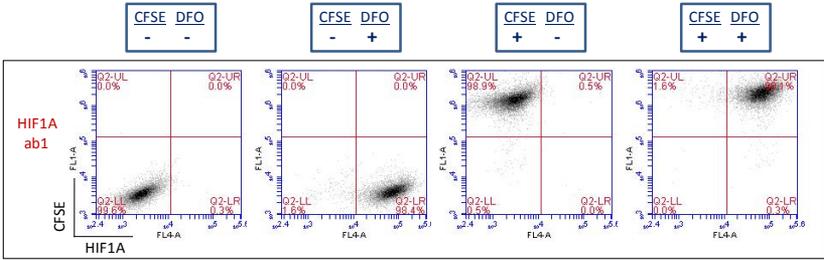


Figure 4. Combination of CFSE labeling and immuno-staining. All combinations of +/-CFSE labeling and +/-DFO treatments were performed on HeLa cells as indicated above each plot. Cells were immuno-stained using an anti-HIF1A antibody. DFO treatment leads to a marked increase in HIF1A intensity as seen in the x-axis shift. +/-CFSE staining is clearly distinguished on the y-axis.

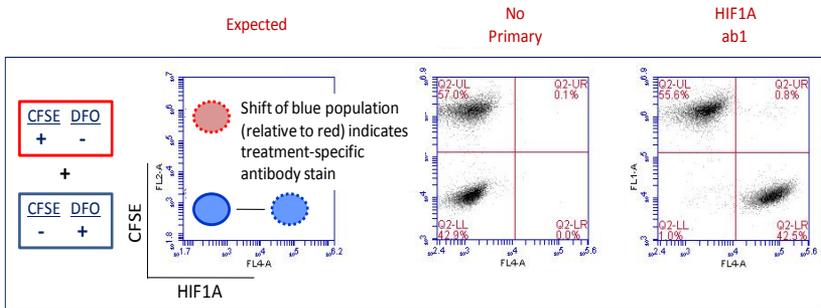


Figure 5. Duplexing treated and untreated cells in a single sample. One plate of HeLa cells was labeled with CFSE and a second plate was treated with the hypoxia mimetic deferoxamine (DFO) but not labeled with CFSE. After harvesting, the cells were combined in a single tube, stained with a HIF1A antibody and subjected to flow cytometry. Expected results are shown in the left panel cartoon. The no primary antibody control sample (middle panel) shows the resolution of CFSE labeled and unlabeled cells. The HIF1A antibody stained cells (right panel) shows that only cells exposed to DFO have an increase in HIF1A protein levels.

Notes

Technical Support

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