TONBO biosciences

Equivalent Performance, Exceptional Value

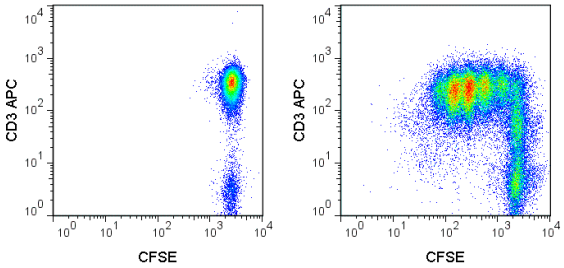
TECHNICAL DATA SHEET

CFSE

Catalog Number: 13-0850

PRODUCT INFORMATION

Contents:	CFSE
Excitation Laser:	Blue (488 nm)
Emission (nm):	521
Use By:	6 months from date of receipt
Storage Conditions:	-20°C protected from light and moisture
Formulation:	lyophilized (500 ug)



Human peripheral blood mononuclear cells were labeled with CFSE (13-0850) and left unstimulated (left panel) or stimulated for 4 days with Anti-Human CD3 and Anti-Human CD28 (right panel). Plots show intensity of CFSE label vs. staining with APC Anti-Human CD3.

DESCRIPTION

CFSE, also known as 5-(and -6)-Carboxyfluorescein diacetate succinimidyl ester, is a non-fluorescent molecule that easily diffuses across cell membranes. Inside the cell, acetate groups are cleaved by intracellular esterases yielding a fluorescent molecule whose succinimidyl ester group covalently interacts with primary amines of intracellular proteins. CFSE is compatible with standard intracellular staining protocols using aldehyde fixation and saponin-based permeabilization. CFSE is widely used to track cell division and to monitor cell migration in vivo. CFSE can be detected using standard fluorescein filter sets by fluorescence microscopy or flow cytometry.

PREPARATION & STORAGE

CFSE is provided as 500 ug of lyophilized powder. CFSE may be reconstituted to a stock concentration of 5 mM with 180 uL of anhydrous DMSO. It is recommended to aliquot smaller volumes and store at -20°C with dessicant and protected from light. Avoid repeated freeze-thaw cycles. CFSE has a molecular weight of 557.47 and after cleavage of acetate groups, it has a peak excitation of 494 nm and peak emission of 521 nm.

APPLICATION NOTES

CFSE (5-(and -6)-Carboxyfluorescein diacetate, succinimidyl ester) has been quality-tested for flow cytometry using stimulated human peripheral blood mononuclear cells. CFSE can be used to label cells at concentrations ranging from 0.5-20 uM, depending on the cell type and assay. It is recommended that the investigator determine the optimal concentration of CFSE for the assay of interest. Over-labeling of cells with high concentrations of CFSE can obstruct normal cell functions and interfere with compensation in multicolor experiments.

REFERENCES

Lyons AB, Parish CR. 1994. J Immunol Methods. 171(1):131-137. Lyons AB. 2000. J Immunol Methods. 243(1-2):147-154. Miller MJ, Wei SH, Parker I, Cahalan MD. 2002. Science. 296(5574):1869-1873. Parish CR, Glidden MH, Quah BJ, Warren HS. 2009. Curr Protoc Immunol. Chapter 4:Unit4.9.

Tonbo Biosciences tests all antibodies by flow cytometry. Citations are provided as a resource for additional applications that have not been validated by Tonbo Biosciences. Please choose the appropriate format for each application and consult Materials and Methods sections for additional details about the use of any product in these publications.

CFSE Labeling Protocol

CFSE Cat. No. 13-0850-U500

***Note**:* CFSE is provided as 500 ug of lyophilized powder. CFSE may be reconstituted to a stock concentration of 5 mM with 180 uL of anhydrous DMSO. It is recommended to aliquot smaller volumes and store at -20°C with dessicant and protected from light. Avoid repeated freeze-thaw cycles.

Other Materials Required

- 1X PBS
- RPMI complete medium (RPMI with 10% FBS, 1% pen/strep, 50 uM 2-ME)

1. Prepare cells as a single cell suspension.
2. Wash cells twice with 1-2 mL 1X PBS to remove serum. Spin at 300-400 x *g* for 5 minutes at room temperature and decant supernatant.
3. Resuspend cells at 1-10 x 10^6/mL in room temperature 1X PBS.
4. Prepare a working solution of CFSE at 2X the desired final concentration in room temperature 1x PBS (ie: for a final concentration of 5 uM CFSE, prepare a 2X working solution by adding 2 uL of 5mM stock to 1 mL 1X PBS).

   ***Note**:* CFSE can be used to label cells at concentrations ranging from 0.5-20 uM, depending on the cell type and assay. It is recommended that the investigator determine the optimal concentration of CFSE for the assay of interest. Over-labeling of cells with high concentrations of CFSE can obstruct normal cell functions and interfere with compensation in multicolor experiments.
5. Add an equal volume of 2X CFSE working solution to cell preparation.
6. Mix immediately and incubate in the dark for 10-20 minutes at room temperature.
7. Quench the labeling reaction by adding 5 volumes of cold complete media and incubate on ice for 5 minutes, protected from light.
8. Centrifuge the cells at 300-400 x *g* for 5 minutes at room temperature and decant supernatant. Wash cells 2 times with 1-2 mL complete media.
9. CFSE labeled cells are ready for assay.