Cellular Proliferation and Tracking With CFDA-SE (CFSE)

Stock and storage:

- Prepare CFDA-SE at a stock concentration 1000-fold higher than the final usage concentration (for example, 2 mM if the final concentration is 2 uM) in dry DMSO.
- Aliquot into single-usage vials and store over desiccant at –20°C.
- CFDA-SE will hydrolyze quickly at room temperature in the presence of water, and much more slowly at –20° under desiccating conditions.
- Aliquoted stocks should be used for no more than 2 months. If your cells show decreased labeling with the same stock of CFDA-SE, hydrolysis is the likely cause.

Method:

1. Suspend cells at in PBS or HBSS containing 0.1 BSA%.
    - Cell concentrations can range widely from 1 x 10^6 cells/mL (for in vitro experiments) up to 5 x 10^7 cells/mL (for adoptive transfer). The cells should be in single cell suspension – if necessary, filter them through nylon mesh immediately prior to labeling.
    - Total reaction volumes should not exceed 4 mL in a 15 mL tube, so prepare cell suspensions at no greater than 2 ml each.
2. Prepare a solution of CFDA-SE from your DMSO stock in PBS/0.1% BSA at 2x the final labeling concentration.
    - For example, if you are labeling at 5 µM, prepare a 10 µM solution. Prepare a volume of CFDA-SE equal to your cell volume above (no more than 2 mL per labeling reaction).
3. Add an equal volume of CFDA-SE solution to your cell suspension. Mix gently and incubate for 5 to 10 minutes at 37°C.
4. Immediately fill the tube with culture (such as complete RPMI (RPMI + 10 % FBS) and centrifuge.
5. Wash the cells two times with tissue culture media at room temperature.
6. Incubate the cells at 37°C for 5 minutes.
    - This allows free, unreacted, CFDA-SE to diffuse out of the cells and be removed in the final wash.
7. For a final time, wash the cells with culture media and then adjust concentration for culturing or other applications.
8. When ready acquire data on a flow cytometer equipped with a 488nm laser and detect CFSE using a 530/30 (or similar) bandpass filter.

Application Notes:

Labeling concentration and conditions:

- Cells are usually labeled at a final CFDA-SE concentration of 0.5 to 5 µM.

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- For best results, do a titration and find the lowest concentration of CFDA-SE that will give effective cell labeling – this will vary from between cell types and also with the application.
- CFDA-SE labeling is somewhat toxic and can induce growth arrest and apoptosis in certain cell types, therefore, it is important to find the lowest acceptable labeling concentration and check the viability after labeling.
- As a rough guide, 0.5 to 2 µM is usually enough for in vitro experiments – cell tracking and generational analysis in transplanted cells may require 2 to 5 µM.
- Incubation time is usually from 5 to 10 minutes – titrate to find the minimal effective conditions.
- Label in PBS or HBSS containing 0.1% BSA.
- All post-labeling washes should be carried out in complete media (such as RPMI with 10% FBS) – your intended tissue culture media is ideal. The high protein concentration inactivates unreacted CFDA-SE.

- When analyzing multi-parameter flow cytometry experiments, CFSE may not be the most optimal choice for tracking proliferation if the green fluorescence channel is required for detecting another antigen/target. In this case, consider using other dyes such as PKH26, Cell Trace Violet, Cell Trace Yellow or Cell Trace Far Red (all obtainable from ThermoFisher – Invitrogen).

References:

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2) Jinyao Chen, Jiao Huo, Zhenchao Jia, Yang Song, Yan Li, Lishi Zhang, Effects of atrazine on the proliferation and cytotoxicity of murine lymphocytes with the use of carboxyfluorescein succinimidyl ester-based flow cytometric approaches, Food and Chemical Toxicology, Volume 76, February 2015, Pages 61-69, ISSN 0278-6915, http://dx.doi.org/10.1016/j.fct.2014.11.026.

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