CFSE staining of the cells

1)- Make a 10^7 cells/ml cell suspension in PBS, BSA 0.1%. I do the staining in a 50ml falcon tube.
If necessary keep an aliquot for FACS.

2)- Add CFSE (from a 10mM stock solution) at a final concentration of 1-10µM depending on the cell type used. For T cells generally between 5 µM and 10 µM are just right.

NOTE: The CFSE stock solutions are kept at -20°C, in small 20µl alicots that are defrosted, used and thrown away. I always put in the box that contains the alicots, some of these granules that catch water because the CFSE is sensitive to humidity.

3)- Keep 10 min at 37°C in the water bath.
If you want to have a stronger staining you can play with the CFSE concentration and the time, and add a couple of more minutes for the staining. But do not exceed 15min.

4)- Add an excess of complete medium FCS 10% RPMI to wash. Complete to 50ml.

Spin at 1500rpm / 5min.

5)- Resuspend the cells in complete medium.
Count the cells again, usually there is 50% loss in the numbers, so do not be surprised.

Spin at 1500rpm / 5min.

6)- Resuspend the cells in RPMI **alone**, to $2x10^6$/200ul → $10x10^6$/ml.
Cells are ready for iv injection.