**CFSE proliferation assay**

1) Resuspend cells in 1X PBS/0.1 % BSA at final concentration of $1\times10^7$ to $5\times10^7$ cells/ml (it can be increased or decreased proportionally).

2) Add 1 to 2 µl of 5mM CFDA-SE* per milliliter of cell suspension (at $1\times10^7$ to $5\times10^7$ cells/ml). Mix up/down. Incubate 15 min at 37°C (water bath). Mix cells every 5 minutes.

3) Quench reaction by adding 1 ml of cold 1X PBS/10% FBS. Spin down.

4) Discard supernatant. Wash 2X with cold culture medium.

5) Count cells. Set up in vitro (culture) or in vivo (adoptively transfer) conditions.

6) Once cell division occurs, follow with extracellular and/or intracellular staining.

**Note:** Alternatively for steps 1 and 2:

1) Centrifuge $1\times10^7$ to $5\times10^7$ cells, discard supernatant and loose pellet but mixing.
2) Add 1 to 2 µl of 5mM CFDA-SE to 1 ml of 1X PBS/0.1% BSA. Mix properly.
3) Add 1 ml of step 2 to cell pellet. Mix up/down.

*5mM CFDA-SE: Dissolve in DMSO at 5 mM (MW$_{\text{CFDA-SE}}$=557; dissolve 2.785 mg/ml). Aliquot and keep in dark/-20°C up to 1 year.*