* KILLING ASSAY WITH BEADS
* label target cells with CFSE.
* optional: label effectors with cell trace violet.
* plate targets+effectors in 96 well U bottom in 5% RMPI: I used 20,000 targets/well and varied the amount of effectors. I used triplicates. No effectors, and E:T ratios of 1:1, 3:1, 9:1 and 27:1. I plated 6 additional wells for maximum killing with targets alone.
* NOTE: I went as low as 5,000 target cell/well and the results were just fine.
* quick spin to have cells slightly pelleted.
* incubate 5 hrs in the incubator 37 C.
* after 5 hrs, spin down, resuspended 3 wells of maximum released with 200 ul 10% bleach or with 10% SDS; and the rest of the wells with flow buffer.
* incubate 5’ RT
* spin down
* stain with viability dye, I like near infra-red.
* wash and spin
* resuspend in 200 ul of flow buffer and add 10,000 beads-APC.
* NOTE: beads are expensive, I used as few as 2,500 beads/well and the results were fine. I tried to keep the ratio between target cells and beads to 2.
* flow 180 ul using HTS plate reader making sure maximum killing are flowed LAST!

note: both bleach and SDS killed all the cells, so it is not even that necessary

How to analyze the data:

Gate on viable target cells and export event count.

Gate on beads and export event count.

For each well calculate the ratio between viable target cells and beads (R)

Average the triplicates.

calculate % specific lysis:

%specific lysis= (R<well of interest> - R<spontaneuos lysis>)/(0-R<spontaneous lysis>) x 100