**Calculations for Cell Capture and Release**

4.6.1) Solution of Ab plus cells: X mL of cells. X µL of Ab. Where X and Y are determined by number of cells needed. For example: For two 8 well plates, the total number of wells with cells needed is 13. At 300,000 cells per well, 3.9 million cells minimum are needed for the experiment. Include excess cells to account for error, so 4.5 million cells. That means X will need to be 2.7, Y will be 1, Z will be 1.8.
If using two glass petri dishes: there is only one solution- mixed cells and Ab solution. In that solution, the solution should comprised of 2.5 mL of cells and 2.5 µL of antibody. If using glass bottom 24 well plates, only one solution is also needed that contains both cells and antibodies, so it will be 3.5 mL of cells, and 3.5 µL of antibody solution.

4.6.2) Ab only solution: Y mL of PBS, Y µL of Ab. Calculate X and Y using same protocol used for the Ab plus cells solution.

4.6.3) Cell only solution: Z mL of cells. Calculate X and Y using same protocol as in the making of the Ab plus cells solution.

4.6.4) Null Solution: Y/2 mL of PBS plus 1% BSA. Calculate X and Y using same protocol used for the Ab plus cells solution.

**4. Antibody biotinylation**

4.1) Remove vial of Sulfo-NHS biotin from -20° C freezer and allow it to equilibrate to room temperature. Dilute the antibody solution to 1 mg/mL. Prepare the 10 mM biotin solution from biotinylation kit in 180 µL of ultrapure water.

4.2) Add 13.2 µL biotin solution to 1 mL of 1 mg/mL antibody solution (Sample calculations below). Incubate solution for 2 hrs on ice (or 30 min at room temperature). Open desalting column by snapping off the bottom and slightly unscrew cap.

4.3) Put the desalting column into a 50 mL conical tube. Centrifuge the desalting column at 1000 g for 2 min. Discard the liquid in the conical tube, making sure to remove the column first to prevent loss of gel or column.

4.4) Dispense PBS into column and centrifuge again at 1000 g for 2 min. Discard liquid in conical tube. Repeat two to three times.

4.5) Place the desalting column into the centrifuge tube. Place the sample on the center of the compacted resin bed in the column. Add the stacker solution if volume is less than 70 µL.

4.6) Centrifuge at 1000 g for 2 min. Discard column. Cap the centrifuge tube and label. Store tube in appropriate fridge following antibody vendor instructions.

NOTE: The final amount of biotinylated antibody should be ~80-90% of starting material.

**4. Antibody biotinylation**

4.1) Remove vial of Sulfo-NHS biotin from -20° C freezer and allow it to equilibrate to room temperature. Dilute the antibody solution to 1 mg/mL. Prepare the 10 mM biotin solution from biotinylation kit in 180 µL of ultrapure water.

4.2) Add 13.2 µL biotin solution to 1 mL of 1 mg/mL antibody solution (Sample calculations below). Incubate solution for 2 hrs on ice (or 30 min at room temperature). Open desalting column by snapping off the bottom and slightly unscrew cap.

4.3) Put the desalting column into a 50 mL conical tube. Centrifuge the desalting column at 1000 g for 2 min. Discard the liquid in the conical tube, making sure to remove the column first to prevent loss of gel or column.

4.4) Dispense PBS into column and centrifuge again at 1000 g for 2 min. Discard liquid in conical tube. Repeat two to three times.

4.5) Place the desalting column into the centrifuge tube. Place the sample on the center of the compacted resin bed in the column. Add the stacker solution if volume is less than 70 µL.

4.6) Centrifuge at 1000 g for 2 min. Discard column. Cap the centrifuge tube and label. Store tube in appropriate fridge following antibody vendor instructions.

NOTE: The final amount of biotinylated antibody should be ~80-90% of starting material.

**Antibody Biotinylation Calculations**

The biotinylation kit comes with biotin reagent to use in conjunction with the antibodies (Ab), however, it is necessary to add only a specific amount of biotin to obtain the intended number of biotin groups on the antibody. A 20-fold excess of biotin reagent is sufficient for 1-10 mg of antibody to have between 4-6 biotin moieties attached to the antibody so it should be more than sufficient for use with the functionalized surface. The number of mM of biotin a 20 fold excess will require must be calculated first. To do this we take:

Next, take the mM of biotin necessary and calculate the amount of µL that would be needed for this concentration. The biotin reagent is reconstituted to 10 mM, so take this concentration to find µL of 10 mM biotin needed to get intended number of mmol biotin:

For example, start with an antibody solution of 1 mL at a concentration of 2 mg/mL, we can calculate the amount of µL needed to add of biotin reagent to get the 20 fold excess.

**HBSS Solution calculations**

Using a hemocytometer, calculate the number of cells in solution. This can be done by counting 3-5 large squares of cells from the hemacytometer, and then averaging the total number of cells. Input them into this formula-

Once number of cells has been calculated, input this into another equation to find the number of mL of HBSS to resuspend the cells in after centrifugation.
:
Using an analytical balance calculate the amount of biotin needed to get to 20 mM solution. First calculate the volume of biotin solution needed. Once the volume is calculated, set up a set of equations to find the number of moles that are needed to get the intended concentration at the intended volume. Using this equation, the necessary number of moles can be calculated:

Once number of moles has been calculated, calculate the number of grams to weigh out on the balance. Using the following equation and the molar mass of biotin which is 244.31 grams :

Hank’s Balanced Salt Solution (HBSS recipe ) (without Calcium, without Magnesium, and without phenol red):
(Adapted from Cold Spring Harbor Protocols

In 500 mL:

4 g NaCl

.2 g KCl

.0402 g Na2PO4\*7H2O

.03 g KH2PO4

.5 g Glucose

Add DI water to get to 500 mL, filter, and then refrigerate.

**Efficiency Equations:**Capture percentages for single cell experiments were calculated via two distinct methodologies- extrapolation and recollection.

Extrapolation uses representative images from the surface to extrapolate total cell pull down and release.








Recollection uses wash step samples counted via hemocytometer or other methodologies.





When using the Fluorescence intensity as a measurement, the background was subtracted out:



We define capture and release percentages this way as:



As such release becomes



By comparing the difference between the extrapolated images as well as the recollection data, the amount of cells destroyed can be estimated.

**Multiple Cell Type Calculations**
For two cell populations the equations are slightly different. The cell of interest is labeled as the positive (+) cell type and the nonspecific cell types are referred to as the negative (-) cell type. Since fluorescence distinction was used to define positive and negative cell types these calculations were done via extrapolation rather than via recollection.

Capture concentration can be written as such:

Release concentration can be similarly written:

We also defined capture and release efficiency as the concentration of positive cell type over the total cell input.





Efficiency of our surface has been quantified using these equations in our Secondary Anchored Cell Release paper.