**Antibody Optimization**

### Introduction:

This protocol is used to determine the total optimal conditions under which to use an antibody.

### Materials:

* Antibodies
* Organ R flies
* BSA block PBS-tween
* BSA block PBS-triton
* Wet Yeast
* Grace’s medium
* Electron Microscopy Science 16% formaldehyde
* 20% lab formaldehyde
* PBS
* PBS-tween (.5%)
* PBS-triton (.2%)

### Procedure:

1. Place Oregon-R flies on wet yeast for 2 days
2. Prepare optimization conditions with which you will test:

* Formaldehyde (16% or 20%)
* Time in formaldehyde (15-20 minutes)
* Detergent (tween or triton)
* Antibody concentration (start at 1:100 for all DSHB)

1. Make a table of all possible combinations of variables. Example:

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Tube 1 | Tube 2 | Tube 3 | Tube 4 | Tube 5 | Tube 6 | Tube 7 | Tube 8 |
| Fix | 16% | 20% | 16% | 20% | 16% | 20% | 16% | 20% |
| Time | 15 | 15 | 20 | 20 | 15 | 15 | 20 | 20 |
| Detergent | Tween | Tween | Tween | Tween | Triton | Triton | Triton | Triton |

**Note:** the number of tubes you will need is the number of variables from each group multiplied. Example:

* Formaldehyde (2 variables)
* Time in formaldehyde (2 variables)
* Detergent (2 variables)
* Antibody concentration (1 variable)

2 X 2 X 2 X 1 = 8

Therefore, you will have 8 tubes per antibody.

1. Prepare optimization tubes with labels for each (be sure to place conditions on labels). Fill each with appropriate type of fix. **Note:** 16% fix is mixed with Grace’s medium (200 ml fix, 400 ml Grace’s), 20% fix is mixed with PBS (100 ml fix, 400 ml PBS)
2. Dissect Organ-R female ovaries (2 pairs/experimental tube).
3. Place in fix for designated time.
4. Rinse with designated detergent.
5. Block with BSA in designated detergent for 20+ minutes.
6. Apply antibodies as normal, continuing with designated detergent throughout protocol. **Note:** Be sure to add a control antibody, such as Fasc III or TJ to samples.
7. Mount slides.
8. Examine slides blindly (as in, without reference to their conditions). Take note of each slide and what you see. **Note:** Pay particular attention to what you see in the FSC area, since this is the cell you are optimizing for.
9. Rank slides in categories by strength – great, medium, poor.
10. Review which slides had which conditions. **Note:** Variable performance may or may not be consistent from slide to slide. For example, triton with longer fix time may work great, while triton with shorter fix time doesn’t show up at all. Don’t be concerned if there is a lack of correlation.
11. Take top 1-3 conditions and retest them, changing antibody concentration as desired.
12. Repeat steps 3-12. After two consecutively strong stains, conditions can be assumed optimal.

### Notes:

* If you are only dissecting 2 pairs/tube, ensure no ovaries are accidently lost in waste disposal during antibody probing.
* To avoid antibody waste, antibodies used at 1:4 can be mixed in as little as 200 ml PBST.
* If staining appears poor, ensure that control antibody staining is still visible.
* Remember, the point of optimizing is to find conditions that work, not to comprehensively exclude every combination that does not work.