DISCOVERIES THAT MAKE A DIFFERENCE

Core Imaging Facility

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Brief Immunogold Protocol
(Non-conjugated primary and conjugated secondary)

1. Prepare a parafilm template using a long strip of parafilm. **Do not touch the surface of the parafilm with your bare hands prior to staining.**

2. Lay down drops of PBS and hydrate grids until ready for next step (~5min).

4. Block with 2% BSA (or blocking solution of choice) for 30 minutes. **May also use 3-5% NFDM or 2º Ab animal serum.**

5. 1 Ab (or PBS for control) for 30-45 minutes. Use at least a 30µl droplet, you may also cover grids and humidify.

6. Rinse 4X, 5 min. each with PBS or dH_2O.

7. 2 Ab** (or PBS for control) for 45 min -1 hour. (α gold; α protein A or G).

8. Rinse 3X, 5 min. each with dH_2O.

9. Post-fix if necessary with 2% PFA 10-15 min.

10. Air dry.

11. Store in grid box.

* Each set of experiments should contain a negative control (the slide is subjected to the same procedure, but instead of adding antibodies all steps receive buffer) and a 2 Ab control (no primary is added, but 2 Ab is added.) If desired, a non-specific primary antibody control may also be performed.

Controls you must have are PBS only; 2º only; and 1º, 2º. You may also have non-1º, 2º; 1º, non-2º, and positive control.