Preparing Antibody-Conjugated Colloidal Gold

Reference: J. W. Slot, H. J. Geuze, A new method of preparing gold probes for multiple-labeling cytochemistry. Eur. J. Cell. Biol. **38**, 87-93 (1985).

1. Heat 40 ml of Reducing Solution and 10 ml of Gold Solution to 60˚C, add together, and stir vigorously.
2. Boil for 5 min. The solution should turn deep red once colloids have formed.
3. Cool on ice and adjust pH to approximately 8.5 with 0.5 M NaOH.

Note: pH paper should be used rather than pH electrodes, which can be damaged by the unstable gold solution.

4. Titrate optimal antibody-gold concentration. Add a range of antibody amounts (0 to 5 μg), in total volumes of 20 μl (in distilled water), to 250 μl of the gold colloid made in steps 1 through 3. Incubate for 5 minutes, RT.

Note: Commercial antibodies usually contain stabilizing BSA or gelatin that will compete with the antibody for gold binding. Affinity-purify the antibody before conjugation.

5. Add 100 μl of 10% NaCl. Look for lowest amount of antibody that stabilizes gold, that is, where no color change to blue occurs (typically 7 to 10 μg/ml gold).
6. Incubate gold with optimal antibody concentration for 10 min.
7. Add pre-spun 10% BSA to a final concentration of 0.1%.
8. Centrifuge to concentrate gold and remove unbound antibody at 120,000*g* for 2- to 3-nm gold, or 100,000*g* for 5-nm gold for 1 hour at 4 ˚C.
9. Collect loose part of pellet. Store gold at 4˚C.

Note: Harvest the pellet as soon as the spin stops to reduce re-suspension of the more compact pellet that contains gold aggregates.

Improved size separation:

1. For further size resolution required for double labeling, load the gold onto a 10 to 40% glycerol gradient prepared with PBS, 0.1% BSA

Note: To make a gradient, carefully pipette an equal amount of each solution into the centrifuge tube working from 40% to 10%. The fraction volume will depend on the size of tube with enough room to load up to 200 ml of gold solution on top.

2. Spin at 200,000*g* (for 2- to 5-nm gold) for 30 min at 4˚C.
3. Collect 20 fractions from the top half of the gradient (approx. 150-300 μl each).

Note: Avoid fractions from the bottom half of the gradient; these contain significant proportions of aggregated gold.

Calibration:

1. Incubate the fractions on a formvar- or pioloform-coated grid for 5 min.
2. Wash the grids twice in 50 μl PBS for 1 min each
3. Add grid to a drop of 50 μl of 2% Methyl Cellulose.

4. Pick up the grids in 5mm copper wire loops, drain excess mixture on filter paper, and leave to dry.
5. Take two images of each fraction in a transmission electron microscope to allow determination of gold size distributions.