

**Colloidal Gold Single Cell Migration Assay**  
**Stack Lab**  
**(from Albrecht-Buehler (1977) Cell 11:395)**

**1. Preparation of cover slips:**

Solutions: (make up fresh for each experiment)

1% bovine serum albumin = 1g/100 ml dH<sub>2</sub>O

Have 1% BSA and 100% ethanol ready in separate beakers for dipping.

1. Grasp coverslip with fine forceps.
2. Completely immerse in 1% BSA.
3. Drain coverslip on paper towel by touching edge of coverslip to towel.
4. Dip coverslip briefly in 100% ethanol. (longer immersion will cause removal of BSA)
5. Again drain coverslip on paper towel by touching edge of coverslip to towel.
6. Place coverslip in well of 12 well tissue culture tray.
7. Repeat procedure for as many coverslips as are needed.

**2. Gold treatment of coverslips:**

Stocks: (store at room temperature)

Sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) = 0.387 g in 100 ml dH<sub>2</sub>O

Gold chloride = 6.85 mg / ml dH<sub>2</sub>O [light sensitive; stored in foil-wrapped container]

Make up fresh:

0.1% formaldehyde = 135 ul in 50 ml dH<sub>2</sub>O. (54 ul in 20 ml dH<sub>2</sub>O)

Have 8 well tissue culture trays containing BSA-coated coverslips ready.

- 1 Combine gold chloride, sodium carbonate, and distilled water in appropriate ratios as indicated below.  
Note that 1 ml of final volume should be enough to treat one coverslip.

For final volume of:	10 ml	20 ml	30 ml	40 ml	50 ml
60 ml					
Sodium carbonate	3 ml	6 ml	9 ml	12 ml	15 ml
18 ml					
Gold chloride	0.9 ml	1.8 ml	7.7 ml	3.6 ml	4.5 ml
5.4 ml					
Distilled water	5.2 ml	10.4 ml	15.6 ml	20.8 ml	26.0 ml
31.2 ml					

- 2 Heat mixture while swirling until boiling. Remove from heat, still swirling.
- 3 Immediately add a volume of 0.1% formaldehyde equal to the volume of gold chloride in the mixture while swirling. The mixture should turn muddy reddish to purplish brown.

- 4 Immediately pipette 1 ml of gold solution onto each coverslip in the tissue culture trays.
- 5 Cover trays and incubate 45 minutes at room temperature or overnight at 4°C.
- 6 Observe with Olympus microscope at 4X (dark field, inverted phase).

### **3. Migration assay:**

- 1 Rinse coverslip once with 1ml HBSS(+). (with Calcium & Magnesium)
- 2 Aspirate and 2 ml of HBSS(+) with 10 ug/ml of CI, incubate at 37°C for 2 hours to allow CI to bind to coverslips.
- 3 Make the cells ready right before time up. Detach the cells with Trypsin and neutralize with Soybean Trypsin Inhibitor. Cells resuspended in SM 1500cells/ml
- 4 Aspirate CI/HBSS, rinse coverslip once with 2 ml of HBSS(-), add 2 ml of each treatment of the cells.
- 5 Incubate the cells in 37°C, 16~18 hours.( depend on the cell type)
- 6 Aspirate, Rinse coverslip once with 1 ml of HBSS(-).
- 7 Aspirate, fix attached cells by add 2 ml of 0.1% Formaldehyde/PBS to well ( can change to PBS after 10 min fix)
- 8 Store the dish at 4°C before analysis.
- 9 Areas of phagokinetics tracks can be evaluated by computer-assisted image analysis