Colloidal gold prep.

Gold sol.

0.5ml $HAuCl_4.2H_2O$

39.5ml dH_2O

Reducing sol.

2ml 1% trisodium citrate

variable 1% tannic acid

variable 25mM K_2CO_3

dH_2O to 10ml

gold size nm	1% tannic acid (ml)	
3.5	2.50	*
4.0	1.25	*
4.5	0.75	*
5.5	0.50	*
6.0	0.25	
7.5	0.13	
10.0	0.04	
11.5	0.025	
14.0	0.013	

* add equal vol. of 25mM K_2CO_3

1. Heat both to 60°C, mix, boil ≥5mins, cool, pH to 8.5 (NaOH) for IgG coupling.

2. Titrate gold-Ab concentration

   μg Ab 0.00 0.25 0.50 0.75 1.00 1.50 2.00 2.50
   add dH2O to 20μl

   add 250μl colloidal gold, mix, incubate 5 mins

   add 100μl 10% NaCl, use lowest concentration where no colour change to blue occurs

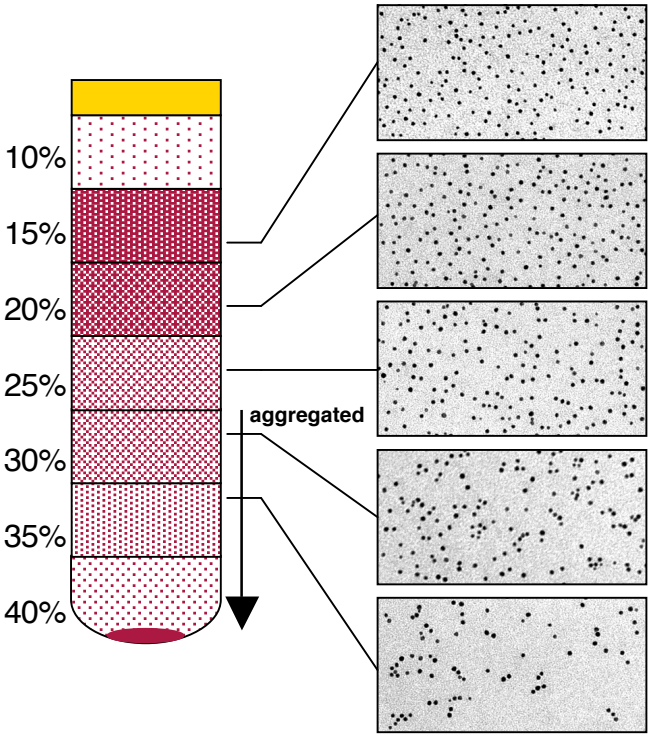
   eg. 1μg in 250μl gold = 4μg/ml gold normally 4-10μg/ml for 5nm gold, more for smaller

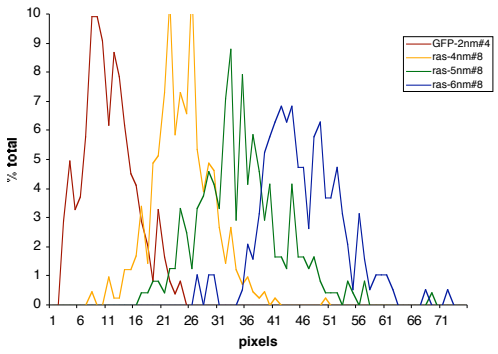
3. Stabilise gold using pre-spun 0.1% BSA final conc.

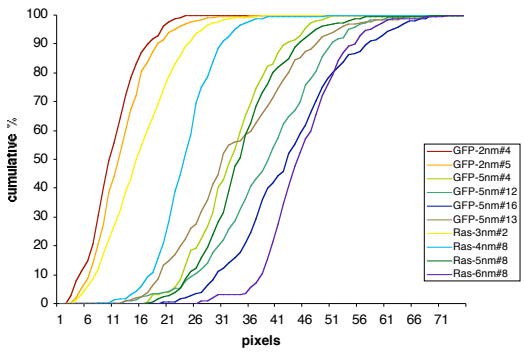
4. Spin to concentrate and remove competing unbound Ab

(120,000g 3nm, 100,000g, 5nm, 50,000g, 10nm, 20,000g, 15nm, 1hour, 4°C)

5. Collect loose part of pellet and load onto 10-40% glycerol gradient (200,000g, 30 min, 4°C) or re-spin in PBS, 0.1% BSA.







1.7ml/layer, 300μl fractions collected