

## Antibody Staining of S2 Cells

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1. Induce cells overnight with 0.7 mM CuSO<sub>4</sub> (Use 8.4 μl of 0.5 M CuSO<sub>4</sub>/6 ml cells).
2. Allow cells to settle on mini glass coverslips (9 mm x 9 mm) in wells of 24-well microtiter dish for 15'.
3. Fix for 10' with 3% paraformaldehyde in PBS. Heat the paraformaldehyde first to depolymerize it.
4. Wash 3 x 10' in PBS.
5. Remove coverslips from wells, wick excess liquid off on a Kimwipe, and place on rubber erasers in plastic box.
6. 1<sup>o</sup> Ab staining: Add 45 μl purified Star Ab diluted 1:2 in BNS or BNN onto cells on coverslips. Allow to stain 60 - 90'. After staining is completed, place coverslips in fresh wells in a 24-well microtiter dish.
7. Wash 6 x 20' with BSS.
8. 2<sup>o</sup> Ab staining for HRP 2<sup>o</sup> (For fluorescent 2<sup>o</sup>, go to step 12):  
Add Goat Rat HRP 2<sup>o</sup> diluted 1:1000 in BNS or BNN to each sample. Incubate 60 - 90'.
9. Wash 6 x 20' with BSS, the 10' in PBS.
10. Incubate samples in 1X DAB diluted in PBS + 5 μl 8% NiCl<sub>2</sub>/ml + 5 μl 3% H<sub>2</sub>O<sub>2</sub>/ml for 10'.
11. Stop reaction by diluting in PBS. Mount samples in glycerol, seal with nail-polish, and store at 4 °C.
12. 2<sup>o</sup> Ab staining for fluorescent 2<sup>o</sup> :  
Add either Goat Rat rhodamine 2<sup>o</sup> or Goat Rat FITC 2<sup>o</sup> diluted 1:250 in BNS or BNN to each sample. Incubate 60 - 90'.
13. Wash 6 x 20' with BSS.
14. Mount samples in Vectashield, seal with nail-polish, and store at 4 °C.

## Antibody Staining of S2 Cells (cont'd)

### Recipes:

#### **BSS:**

per liter:

2.21 g NaCl  
3.98 g KCl  
3.07 g MgSO<sub>4</sub>-7H<sub>2</sub>O  
0.74 g CaCl<sub>2</sub>-H<sub>2</sub>O  
1.79 g tricine  
3.60 g glucose  
17.12 g sucrose ]  
2.00 g BSA

Add ddH<sub>2</sub>O to 900 ml, adjust pH to 6.95, filter-sterilize, and store at 4 °C.

#### **BNS:**

BSS + 10% normal goat serum + 10 mg/ml saponin  
(always make fresh)

#### **BNN:**

BSS + 10% normal goat serum + 0.4 % NP-40  
(always make fresh)