

Antibody Staining of S2 Cells

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1. Induce cells overnight with 0.7 mM CuSO₄.
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.
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°-Ab staining: Add 45 L 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° Ab staining for HRP 2° (For fluorescent 2°, go to step 12):
Add GoataRat HRP 2° 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 L 8% NiCl/ml + 5 L 3% H₂O₂/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° Ab staining for fluorescent 2° :
Add either GoataRat rhodamine 2° or GoataRat FITC 2° 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.

Recipes:

BSS:

per liter:

2.21 g NaCl
3.98 g KCl
3.07 g MgSO₄-7H₂O
0.74 g CaCl₂-H₂O
1.79 g tricine
3.60 g glucose
17.12 g sucrose
2.00 g BSA

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)

Add ddH₂O to 900 ml, adjust
pH to 6.95, filter-sterilize, and store at 4 °C.