Working Protocol for BrdU detection in thin plastic sections. [PFA 4% fixed samples]

1. Shake stock Sodium Ethoxide gently to mix. Solution should be dark to ensure removal of **all** plastic; Immerse slides in Sodium Ethoxide diluted 1:4 in Ethanol.
   -Allow at least 7-10 min for 250 nm thick sections at room temperature.
2. Wash 3 x 2 min. in fresh methanol/ethanol.
3. Wash 5 min. in a **cool** tapwater rinse. Dip in ddH_2O and dry slides gently.
4. Digest desired sections with **FRESH** 0.15% Trypsin in 0.05 M Tris/HCl buffer with 0.1% $CaCl_2$ at pH 7.6, for 12 min at room temperature (other sections not receiving the primary anti-BrdU will not receive trypsin).
   -wash 2 min. in 0.1 M Phosphate Buffer
   -dip ddH_2O and dry. Do not allow the PB to dry on sections!
5. Apply primary antibody (Mouse anti-BrdU) 1:100 in 1% goat serum with 0.3% Triton X, 0.9% NaCl, but plain 1-3% GS will work as well. Incubate 2 hrs at room temperature or over night at 4°C. To avoid drying, place slides on a wetted paper towel and cover with a lid. Place a plastic wrap over the system to keep moisture in.
6. Wash slides for 30 min. in 0.1 M PB.
   -dip ddH_2O and dry.
7. Apply respective secondary antibodies. Be sure to use proper antibodies. For BrdU sections, use Goat anti-Mouse 1 nm Gold diluted 1:50 in 1% goat serum with 0.3% Triton X, 0.9% NaCl.
8. Incubate at RT for 1 hour. Be sure to prevent drying of secondary antibody.
9. Wash 1 hour in 0.1 M PB.
   -dip ddH_2O and dry gently.
10. Silver intensify using the following method (Marc Lab protocol):

Silver intensification solutions

- Stock A = 114 mg citric acid + 342 mg sodium citrate in 6 ml deionized water (make fresh - do not store)
- Stock B = 0.5 g hydroquinone in 15 ml deionized water (make fresh - do not store)
- Stock C = 1% aqueous silver nitrate (may be stored indefinitely at room temperature wrapped in foil)
- Working solution = 5 ml A + 1 ml B + 1 ml C, in that order, made up immediately before use
- Stop solution = 5% acetic acid
- Optional: Slide warmer set at 30°C (Avoid cold surfaces. Temperatures below 24°C slow the reaction).

Procedure

1. Prepare silver intensification solutions. Stock solution C may be prepared in advance. Prepare stock solutions A and B separately prior to intensification and use within 1 hour.
2. Use the working solution immediately as it lasts only 10 min. Add 25-50 microliters of solution per well in quick succession; a small disposable dropper works best. (Alternative: make 15 ml of working solution and process in a plastic slide cassette). Expose sections to the solution for 4-8 minutes in a dark location, such as under an aluminum cover. You needn't work in absolute darkness, but the background decreases slightly if the intensification occurs away from bright light. The reaction is temperature sensitive and slows substantially below 24°C.
3. Stop the reaction with a brief dip in 5% acetic acid.

4. Wash for 10 minutes in deionized water and dry with an air canister.
5. Cover slip in a medium of your choice.

Working Protocol for BrdU detection in thin plastic sections. [GA 2.5%/1% FA -fixed samples]

1. Shake stock Sodium Ethoxide gently to mix. Solution should be dark to ensure removal of **all** plastic; Immerse slides in Sodium Ethoxide diluted 1:4 in Ethanol.
   -Allow at least 7-10 min for 250 nm thick sections at room temperature.
2. Wash 3 x 2 min. in fresh methanol/ethanol.
3. Wash 5 min. in a **cool** tapwater rinse. Dip in ddH_2O and dry slides gently.
4. Digest desired sections with **FRESH** 0.15% Trypsin in 0.05 M Tris/HCl buffer with 0.1% $CaCl_2$ at pH 7.6, for 20 min at 37°C (other sections not receiving the primary anti-BrdU will not receive trypsin).
   -wash 2 min. in 0.1 M Phosphate Buffer
   -dip ddH_2O and dry. Do not allow the PB to dry on sections!
5. Apply primary antibody (Mouse anti-BrdU) 1:100 in 1% goat serum with 0.3% Triton X, 0.9% NaCl, but plain 1-3% GS will work as well. Incubate between 2 hrs and over night at room temperature. To avoid drying, place slides on a wetted paper towel and cover with a lid. Place a plastic wrap over the system to keep moisture in.
6. Wash slides for 30 min. in 0.1 M PB.
   -dip ddH_2O and dry.
7. Apply respective secondary antibodies. Be sure to use proper antibodies. For BrdU sections, use Goat anti-Mouse 1 nm Gold diluted 1:50 in 1% goat serum with 0.3% Triton X, 0.9% NaCl.
8. Incubate at RT for 1 hour. Be sure to prevent drying of secondary antibody.
9. Wash 1 hour in 0.1 M PB.
   -dip ddH_2O and dry gently.
10. Silver intensify using the following method (Marc Lab protocol):

Silver intensification solutions

- Stock A = 114 mg citric acid + 342 mg sodium citrate in 6 ml deionized water (make fresh - do not store)
- Stock B = 0.5 g hydroquinone in 15 ml deionized water (make fresh - do not store)
- Stock C = 1% aqueous silver nitrate (may be stored indefinitely at room temperature wrapped in foil)
- Working solution = 5 ml A + 1 ml B + 1 ml C, in that order, made up immediately before use
- Stop solution = 5% acetic acid
- Optional: Slide warmer set at 30°C (Avoid cold surfaces. Temperatures below 24°C slow the reaction).

Procedure

6. Prepare silver intensification solutions. Stock solution C may be prepared in advance. Prepare stock solutions A and B separately prior to intensification and use within 1 hour.
7. Use the working solution immediately as it lasts only 10 min. Add 25-50 microliters of solution per well in quick succession; a small disposable dropper works best. (Alternative: make 15 ml of working solution and process in a plastic slide cassette). Expose sections to the solution for 4-8 minutes in a dark location, such as under an aluminum cover. You

needn't work in absolute darkness, but the background decreases slightly if the intensification occurs away from bright light. The reaction is temperature sensitive and slows substantially below 24°C.

8. Stop the reaction with a brief dip in 5% acetic acid.
9. Wash for 10 minutes in deionized water and dry with an air canister.
10. Cover slip in a medium of your choice.