

## BrdU Labeling in Ovaries

1. Dissect ovaries in non-supplemented Grace's medium (Gibco BRL) at RT and partially tease apart ovarioles. Do not put the tissue on ice, as this appears to affect incorporation in some cell types.
2. Remove medium and add 1ml 10micromolar BrdU (Sigma) ( $1:10^3$  from frozen 10mM stock) in Schneider's medium. (\*Put 1-2 $\mu$ l BrdU in 1 ml Schneider's\*). Mix completely and incubate for one hour with rocking, in BSA-treated microcentrifuge tube.
3. Rinse several times with EBR (or PBS) to remove unincorporated label and fix for 15-20 min in formaldehyde/Buffer B/dH<sub>2</sub>O (1:1:4). (\*6% Formaldehyde 200 $\mu$ l form./1000  $\mu$ l PBT\*)
4. Wash with PBT 3 x 5min (\*Rinse and spin\*)
5. Acid treat for 30 min (\*35-40 min\*) in 2N HCl, fresh. Take 12N HCl and dilute it to 2N, adding a drop of tween. (\*The HCl is yellow if it has gone bad. This step cuts off pyrimidine bases. Alternatively, use mild DNase treatment\*)
6. Neutralize in 100mM borax (Na tetraborate) for 2 min.
7. Wash with PBT 3 x 10min. (\*quick washes/rinses optional\*)
- 8 Block with PBT/5% normal goat serum (NGS) 1/2 hr.
9. Incubate 1 hour at room temperature in PBT/NGS with 1:100 anti-BrdU monoclonal Ab (Becton Dickinson Cat. #7580). Make it 5% NGS (500 $\mu$ l)
10. Rinse 3 x 10 minutes in PBT.
11. Incubate in secondary antibody 1:400 G $\alpha$ M-Cy3 5%NGS in PBT for 1 hour at RT.
12. Remove the secondary antibody. Add DAPI (2 mg/ $\mu$ l)(1:1000 in PBT) for a minute.
13. Wash 3 x 10 minutes in PBT.

**BrdU is a mutagen and should be handled with care.** Aliquots are OK through at least 2-3 freeze/thaw cycles.

**Schneider's needs to be fairly new.**

2N HCl=8.6 ml of concentrated stock/50 ml

Borax Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> · 10H<sub>2</sub>O 1.907g/50ml

Keep your borax stock at 4 C to prevent mold growth.

PBT PBS + 0.1% Triton X-100

**Buffer B**

KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub> (pH 6.8) 100mM

KCl	450 mM
NaCl	150mM
MgCl <sub>2</sub>	20mM