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BrdU Labeling & Antibody Detection of Mouse and Chicken Embryos

Note: BrdU is a mutagen and can be toxic. Wear gloves when handling BrdU and dispose of all liquid and solid waste as hazardous waste. See the UCLA fact sheet for more details

Mice:

1. Prepare 5 mg/ml solution of BrdU (Sigma) in PBS. Filter through a 2 µm filter.

2. Weigh pregnant mouse (I usually do this by taring an empty 600 ml or 1 L beaker on the balance and then placing the mouse in the beaker).

3. IP Inject the mouse with a volume of the BrdU solution based on the weight of the animal: 100 µg of BrdU per g of body weight (comes out to 0.02 ml of 5 mg/ml BrdU solution per g of body weight).

4. Sacrifice the animal after 2 hours. You can sacrifice the animal sooner if you want to label fewer cells. However, I don't know if waiting longer will get you much, as the BrdU doesn't stay very long in the mouse's circulation (it gets taken up by the mother's tissues and not much is left for the embryos). If you want to label more cells, then you will have to do repeated BrdU injections.

5. Process the embryos as you normally would for either antibody or in situ analysis.

Chicks:

1. Prepare a 1 mM solution of BrdU (Sigma) in L15 media (PBS or other TC media is probably fine). Filter through a 0.2 µm filter. I often store this solution wrapped in foil at 4°C for a month or two or indefinitely at -20°C.

2. Open egg and tear a hole in the vitelline membrane overlying the embryo with a sharpened tungsten needle.

3. Carefully add 200 µl of the BrdU solution to the embryo, trying to get the solution underneath the vitelline membrane.

4. Cover over the hole in the egg with parafilm and return it to the incubator. Incubate for ~30 minutes before extracting the embryos. If you want more extensive labeling, you can incubate the BrdU-treated embryos longer. However, I suspect that the standard dose of BrdU used may be toxic to the embryos over time, and that you should significantly decrease the amount of BrdU for long labeling procedures.

5. Process the embryos as you normally would for either antibody or in situ analysis.

Antibody detection:

1. If you are going to do double (or triple staining), stain your sections for the other antigens (including the secondary antibody staining step) first.
2. Dip the slides in 4% paraformaldehyde in PBS (or PB; I generally use left-over fix that I've accumulated on my shelf) for 5-10 minutes. Wash the slides briefly with PBS-T.
3. Immerse the slides in a solution containing 4N HCl, 0.1% Triton X-1000 for 5 minutes. If you find that you are getting horrible disruption of nuclear morphology, you can try dropping the HCl concentration down to 2N.
4. Remove the slides and wash several times with PBS-T. Leave the slides in one of these washes for at least 5 minutes. Some protocols include a neutralization step where the slides are immersed in a sodium borate solution. I personally don't bother with this. Several changes of PBS-T seem to be adequate for neutralization.
5. Overlay the slides with blocking solution. This really isn't to block the tissue, but to equilibrate the slides before adding the BrdU antibody.
6. Incubate the slides for at least 45 minutes at RT˚C with a BrdU antibody (overnight staining at 4˚C is also fine and may be more sensitive). I've successfully used three: one is a FITC-BrdU mouse monoclonal (direct conjugate of FITC to the antibody) from Becton-Dickinson (Cat. # 347583), diluted 1:25; another other is a rat monoclonal (unconjugated) from Harlan Sera-Lab Ltd. (available through Accurate Chemical Co.) (Product # MAS-250p [purified antibody, 0.5 mg/ml], the antibody clone is called BU1/75 (ICR1)and may be sold by other vendors), diluted 1:2000; and the third is a mouse monoclonal called G3G4 from the Developmental Studies Hybridoma Bank, hybridoma supernatant diluted 1:50. Note that if you use the unconjugated BrdU antibodies, then you must use a secondary for these.
7. Immerse the slides in (or overlay the slides with) 4% paraformaldehyde again. If not fixed, the BrdU staining will often "diffuse" into the mounting medium, and obscure your analysis. (note this diffusion seems to be most attributed to the use of certain mounting medias (i.e. Vectashield). The fixation step may not be necessary with other mounting medias.
8. Coverslip slides with Prolong gold mounting media (Invitrogen).