

Topical Application of BrDU; Meg Winberg, Stellar Lab

Stock solution: BrDU is dissolved in DMSO. The solution freezes at 4°C, which seems to have some detrimental effect. To get around this I have been adding acetone to the stock as an antifreeze. My stock ends up as 100 mg BrDU per ml 1:1 DMSO:acetone. I have been making up only 100-100 µl at a time, which has been stable when stored in the fridge for up to a month.

Working solution: Dilute in acetone immediately before use. This solution is quite volatile and doesn't last long; I use the smallest tubes possible and close them to reduce evaporative loss. I have found that a final concentration of 1 mg/ml will label 5 to 6 rows of dividing cells in third instar eye discs, giving strong labelling in the optic lobe OPC and IPC and a narrow band in the lamina.

Treatment: Larvae are collected, washed and treated batchwise. I find that it works best to dry the larvae by putting the corner of a paper towel in the PBS puddle. "Dried" (not desiccated!) larvae are piled up and BrDU:acetone is dropped onto them. I then stir the solution and larvae until the acetone dries. I have been using 7 µl for 20 second instars or 10 µl for 20 third instars. After treatment the larvae should be mobile and active; if not you should try less acetone. Morbidity is the result of acetone soaking!

Post treatment: Larvae are transferred to fresh food for the chases. I find it helpful to stir up the food first, so the animals don't have to work so hard to get into it.