

#### HOW TO MAKE A VITAL STAIN

Prepare 25 ml of 2 % agar (.5 g agar powder, 25 ml water) bring to a boil gently so agar all dissolved. Divide into 2 parts, add 25 mg of Nile Blue Sulfate to one batch and dissolve; add 25 mg neutral red to the other half and shake to dissolve. pipette 1 ml of these agars onto microscope slides and allow to harden. Place into racks so they will dry over the next few days.

To be used: place a drop of culture medium on the agar slide, after 2 min cut off pieces of agar the size you want and place them in the medium you will be using on the embryos and allow dye to diffuse out until you can't see it coming out anymore. Redry agar slide for later use on chick embryos

You can place the dye alternating colors in a row or just one place with only one dye. Then place embryo into wax dish depression, place agar dye pieces in position and cover with a bent piece of cover slip or pieces of foil stuck into the wax to hold all in position. Draw before and after Stain for 20-30 min. Good stages to use are gastrula, neurula, tailbud (need anesthesia) in frog; primitive streak, neural folds, limb bud in chick probably needs to be done in saranwrap culture or glass ring culture.

Keep all results after preserving in formaldehyde, then dissect them to see the results.