

## **ALCIAN BLUE STAIN FOR XENOPUS TADPOLES**

This takes a week or more. Vertebral cartilages begin to appear around time of limb bud development. "Overfeeding" with frequent water changes at RT speeds up time to metamorphosis to ~2-3 weeks.

### **FIX:**

Tadpoles at least overnight in several changes of Ethanol. (Formaldehyde fix at this stage traps a lot of stain in soft tissues, it is better to fix again after staining for greater integrity of the soft tissue). Embryos can be stored @ -20°C in Ethanol.

### **STAIN**

Transfer embryos from Ethanol into Alcian Blue stain:

0.1mg/ml Alcian Blue in 1 part acetic acid: 4 parts Ethanol

Stain for 3-4 days at RT.

### **DIFFERENTIATE**

Do several long rinses (hr+) in 1% HCl in 70% Ethanol and then overnight for 1-2 days until staining of soft tissues subsides. You should be able to make out that the vertebral cartilages are stained

Rinse several times in Ethanol (95-100%)

### **REFIX**

Hydrate by steps (20min or more each):

50% Ethanol: 50% H<sub>2</sub>O

25% Ethanol: 75% MEM

MEM

Fix in MEMFA 1-2 hours to maintain integrity of the embryo (this may not be necessary although delicate embryos may fall apart in the maceration step if it is not done).

Rinse in 0.5X SSC several times

### **BLEACH**

If embryos are pigmented, bleach under fluorescent lights with:

1% H<sub>2</sub>O<sub>2</sub>, 5% formamide in 0.5xSSC

Rinse with 0.5xSSC

### **MACERATE**

Do several changes of 2% KOH over the course of a day (this removes additional background).

### **CLEAR**

Do several rinses with 50% Ethanol: 50% Glycerol, until solution is no longer blue, then allow to clear overnight- several days.

After several days transfer through:

60% glycerol in 2% KOH

80% glycerol in 2% KOH

Wait until embryos sink in both, several changes may be required as additional stain is removed. Embryos can be stored in 80% glycerol in 2% KOH.