Alcian Blue/Alizarin Red skeletal prep. Protocol

Starting with a formaldehyde-fixed specimen that has been dehydrated into 70% EtOH:

1. Transfer the animal in Alcian Blue solution (overnight).
2. Transfer the animal into 70% EtOH (~2 hrs or until the animal sinks).
3. Transfer the animal into 50% EtOH (~2 hrs or until the animal sinks).
4. Transfer the animal into 25% EtOH (~2 hrs or until the animal sinks).
5. Transfer the animal into diH2O (~2hrs or until the animal sinks).
6. Incubate the animal in Alizarin red solution overnight.
7. Soak the animal in a solution of 1% (w/v) trypsin (Fisher T360-500) in 2% (w/v) sodium tetraborate until clear. Watch carefully! – it can take anywhere from 15 min to several hours/days, depending on the size of your animal. [For a skate hatchling, you can do the trypsin step overnight]
8. Transfer the animal into 3:1 0.5% KOH:glycerol (24 hrs to several days, depending on the size of your animal. Change this solution daily).
9. When the animal is sufficiently clear, grade the animal in glycerol (1:1 0.5% KOH:glycerol, 1:3 0.5% KOH:glycerol, 80% glycerol for 24 hrs each).

Solutions

Acetic ethanol
- 30mL glacial acetic acid
- 70mL 100% EtOH

Alcian blue solution
- 20mg Alcian Blue
- 100mL Acetic ethanol

Alizarin red solution
- 0.1g of Alizarin red
- 100mL 1% KOH