

Clearing and Staining for bone and cartilage
adapted from Dingerkus and Uhler 1977

Procedure:

1. Fix fresh material in 10% formalin 2-3 days or longer. Specimens already in formalin or alcohol, ignore step 1 and go to step 2.
2. Wash in several changes of distilled water for 1-2 days.
3. Place directly into a mixture of 10 mg Alcian Blue 8GN (supplied by Matheson, Coleman and Bell), 80 ml 95% ethyl alcohol and 20 ml glacial acetic acid 10-12 hours. (This solution must be fresh)
4. Transfer to two changes of 95% ethyl alcohol, 1-2 hours per change.
5. Transfer through an alcohol series of 75%, 40%, 15% ethyl alcohol 2-3 hours in each, or until specimen sinks.
6. Transfer to distilled water 2-3 hours, or until specimen sinks
7. Place in an enzyme solution of 30 ml saturated aqueous sodium borate, 70 ml distilled water, and 1 g trypsin enzyme. Change solution every 2-3 days, or sooner if solution turns a bluish color. Continue until bones and cartilage are clearly visible, and the flesh retains no blue color. NOTE: the skin of some gobies remains blue at this stage.
8. If bone is present: Transfer to 0.5% aqueous KOH solution, to which enough Alizarin Red S stain has been added to turn solution a deep purple-red. Leave 24 hours or until bones are distinctly red. If bone is not present go to step 9
9. Transfer through a 0.5% KOH - glycerin series of 3/4 KOH - 1/4 glycerin, 1/2 KOH - 1/2 glycerin, 1/4 KOH - 3/4 glycerin, and finally to pure glycerin.
10. Store specimen in pure glycerin to which a few crystals of thymol have been added to inhibit molds and bacteria.