

Embedding protocol modified from Humason (1979).

Start: Specimens fixed in 10% neutral buffered formalin and post-fixed in 70% ethanol.

H ₂ O with 2 changes.....	2 hours
Decalcify in Formical-2000™.....	time variable
Smallest skull (10.1 mm long) decalcified in 20 hours	
Largest skull (22.7 mm long) decalcified in 136.5 hours	
Soak in 70% ethanol.....	1 hour
Soak in 95% ethanol.....	2 hours
Soak in 100% ethanol.....	1 hour
Soak in 100% ethanol.....	1 hour
Soak in 100% ethanol.....	1 hour
Soak in xylene.....	1 hour
Soak in xylene.....	1 hour
Soak in melted paraffin at 60° C.....	1 hour – overnight
Soak in melted paraffin at 60° C.....	1 hour

Transfer tissue to mold. Mold should have mold-release applied and dried to the inside. Apply thin layer of paraffin at bottom of mold, let partially solidify, place and orient tissue as desired, and fill the rest of mold with melted paraffin. Let solidify at room temperature.

Staining protocol modified from Garvey (1984).

Start: Sections embedded on glass slides at 10 µm thick and cured on a 40° C hotplate overnight.

Preparing Solutions:

Biebrich Scarlet-Acid Fuchsin Solution

 1% aqueous Biebrich Scarlet, 180 ml

 1 % aqueous Acid Fuchsin, 20 ml

 Glacial Acetic Acid, 2 ml

Light Green Solution

 Light Green, 5 g

 Deionized H₂O, 250 ml

 Glacial Acetic Acid, 2 ml

Phosphomolybdic-Phosphotungstic Acid Solution

 5% Phosphotungstic Acid, 25 ml

 5% Phosphomolybdic Acid, 25 ml

Deparaffinize:

CitriSolv™.....3 minutes

CitriSolv™.....3 minutes

CitriSolv™.....3 minutes

Hydrate:

100% ethanol.....	2 minutes
100% ethanol.....	2 minutes
95% ethanol.....	2 minutes
70% ethanol.....	2 minutes
Deionized H ₂ O.....	2 minutes
Stain:	
Mayer's Hematoxylin.....	1 minute
Rinse:	
Warm running tap H ₂ O.....	10 minutes
Stain:	
Biebrich Scarlet-Acid Fuchsin Solution.....	15 minutes
Rinse:	
Deionized H ₂ O.....	4–5 dips
Differentiate:	
Phosphomolybdic Acid-Phosphotungstic Acid Solution.....	16 minutes
Rinse:	
Deionized H ₂ O.....	2–3 dips
Stain:	
Light Green Solution.....	5 minutes
Rinse:	
Deionized H ₂ O.....	2–3 dips
Differentiate:	
5% Phosphotungstic Acid.....	1 minute
Rinse:	
Deionized H ₂ O.....	1–2 dips
Deionized H ₂ O.....	1–2 dips
Dehydrate:	
95% ethanol.....	1 minute
100% ethanol.....	40 seconds
100% ethanol.....	40 seconds
Clear:	
CitriSolv™.....	3 minutes
CitriSolv™.....	3 minutes
CitriSolv™.....	3 minutes

Mount with Permount™, apply glass coverslip, and cure on a 40° C hotplate overnight.