Paraffin Embedding of Cartilaginous tissues
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Updated on 12/14/11

Reagents:

1) 10% Phosphate Buffered Formalin (FisherSci, Cat #SF100-4)
2) Immunocal™ mild formic acid decalcifier (Decal Chemical Corp, Cat # 1440QT)
3) Graded Ethanol solutions in ddH_20 or DEPC H_20: 50, 70, 80, 90, 100%
4) Xylenes (HistoPrep, FisherSci, Cat#HC-700)
5) Paraffin (McCormick Paraplast, FisherSci, Cat#12-646-106)
6) Paraffin blocks (flat bottom or tapered)

Equipment: Heated vacuum oven (65-70°C; pressure at approx. 40 cmHg)

Procedure:

1) Dissect tissue (forelimbs, hindlimbs, vertebral columns) from the embryo and place in a glass tube with 1-2ml 10% Phosphate buffered formalin. (If using a whole embryo, expose the chest cavity or eviscerate for maximal fixation.) Fix samples at 4°C overnight with shaking for a maximum of 16 hours.
2) Rinse the samples in ddH20 3x 10min.
3) Immerse samples in Immunocal™ and decalcify at 4°C:
    a. For postnatal day 0 and embryonic samples- overnight
    b. For adult bones (6 weeks and older), 3 days max
4) Rinse the samples in ddH20 3x 10min.
5) Dehydrate samples in 50-100% EtOH solution overnight at 4°C with each solution.
6) Clear the samples in 3 changes of Xylene over the course of a day. After the last xylene change, add about 50% heated paraffin to samples. Place samples in heated vacuum oven overnight.
7) The next day, perform 2 changes of paraffin and embed samples in paraffin blocks.