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We use this protocol and it's working

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Bone Decalcification Protocol Using 14% EDTA Buffer Solution pH 7.2 - 7.4

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ABSTRACT

This protocol describes the steps required for successful decalcification. Decalcification describes the technique for removing minerals from bone or other calcified tissue so that good-quality paraffin sections can be prepared that will preserve all the essential microscopic elements. Decalcification is carried out after the specimen has been thoroughly fixed and prior to routine processing to paraffin.

	A	B	C
	10% Buffered Formalin	Fisher	STL286001
	Ethylenediaminetetraacetic (Acid free EDTA)	VWR	97061-404
	Ammonium hydroxide	Fisher	1336216
	Phosphate Buffered Saline	Gibco	10010-023

Preparation of Ethylenediaminetetraacetic acid (EDTA)

1 Make 14% EDTA solution fresh

a. Add 140 g free acid EDTA to 700 mL distilled H2O

b. On stir plate in the fume hood, add ammonium hydroxide, 30 mL at a time, until solution clears (about 90 mL total)

c. Add H2O to almost 1 L. Check pH and adjust with ammonium hydroxide dropwise up to pH 7.4, then adjust final volume to 1 L

Procedure

3d

2 Dissect bone and remove as much soft tissue as possible.

3 After appropriate fixation in 10% buffered formalin - 24:00:00 - 48:00:00 hours, wash tissue in distilled H2O or used EDTA solution. 3d

4 Place tissue in 14% EDTA solution at 4 °C C on a stirring device to circulate the EDTA. Use enough solution to saturate tissue (fluid volume to tissue ratio is critical for the decalcification process). The EDTA solution should be at least 20X the volume of the tissue to ensure proper decalcification.

> Note
>
> Fluid volume to tissue ratio is critical for the decalcification process.

5 Periodically check bone for adequate decalcification. Refresh EDTA solution daily for first 5 days, then may leave in same EDTA solution without changing.

6 Decalcification is complete when bone is soft and pliable. This may take 10 days or more depending on tissue size. Can check by X-ray, or probe with a needle and/or bend tissue to determine if tissue is soft enough to section. Over-decalcification will cause tissue or cells to lose affinity for certain stains.

7 Rinse once with Phosphate Buffered Saline (PBS) for 00:30:00 minutes 30m

8 Rinse twice in ddH$_2$0 for 00:30:00 minutes 30m

9 Place in 70% Etanol (EtOH). Store at 4 °C (no more than 2 weeks)

10 Proceed with tissue processing