EDTA decalcifying solution (pH 7.2)

Introduction

When tissues contain bone or calcified lesions, calcium-containing tissues are not suitable for direct paraffin embedding and sectioning. This is due to the difference in density between calcium and paraffin, which makes it difficult to obtain complete sections. For calcium-containing tissues, it is best to fix the tissue first, then decalcify, or perform both simultaneously. Subsequent steps include dehydration, clearing, paraffin infiltration, embedding, and sectioning. Various reagents are available for decalcification, including organic acids, inorganic acids, ethylenediaminetetraacetic acid (EDTA), and electrolytic decalcification.

EDTA is a relatively good chelating decalcifying agent that causes minimal damage to tissue structure and preserves certain enzymes in the tissue. Tissues decalcified with EDTA can be used for immunohistochemistry and in situ hybridization staining. However, this method is very slow, typically taking several weeks to months.

Protocol

1. For bone tissue decalcification, the specimen should not be too thick; approximately 5 mm is recommended.
2. After tissue fixation, wash three times with PBS, 20 minutes each time.
3. Wash the tissue three times with distilled water, 20 minutes each time.
4. Transfer the tissue to 20–30 volumes of EDTA decalcification solution. Decalcify for 10–30 days or longer. To accelerate decalcification, perform the procedure at 37°C. If necessary, replace with fresh EDTA decalcification solution and continue decalcification. Most tissues require 2 weeks to 3 months, with the solution changed once a week until the endpoint is reached. Alternatively, a microwave-assisted rapid decalcification method can be used: Set the microwave to approximately 200 W, heat for 5 minutes each time, repeat 3–5 times depending on tissue thickness and density, with 3–5 minute intervals between heating cycles.
5. Rinse several times with distilled water.
6. Proceed with routine dehydration and embedding.

Note

1. For bone tissue blocks 5 mm thick, decalcification typically takes 10–30 days.
2. Moderate heating can accelerate decalcification, but the temperature should generally not exceed 37–40°C. Excessive heat may cause the bone tissue to loosen or disintegrate, and temperatures above 60°C must be

avoided.

3. Decalcification must be complete, avoiding both incomplete and excessive decalcification. The degree of decalcification should be controlled to minimize the decalcification time while ensuring no adverse effects on tissue sectioning, as prolonged decalcification may cause tissue damage.
4. Avoid using metal containers for decalcification; use glass containers whenever possible.
5. Bone tissue decalcification should be performed after fixation or simultaneously with fixation, not before fixation, to minimize tissue damage.
6. Check the degree of decalcification periodically to avoid over-decalcification, which increases tissue damage and affects staining results.
7. Determination of the decalcification endpoint (physical method): Use methods such as needling, pinching, or clamping. When the bone tissue becomes soft or no resistance is felt upon needling, decalcification can be terminated. Physical detection methods may cause some damage to tissue structure; avoid excessive force or repeated testing.
8. Storage conditions: Store at room temperature; valid for 1 year.
9. This product is for scientific use only.

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