Protocol For Zinc Fixation:

1. Prepare 0.1M Tris buffer, pH 7.4 (IL) Tris Base 12.1 g (THAM, TRIZMA) Deionized water 900 mls 1.o N HCL 81.5 mls

2. Prepare Zinc-Fixative as follows:

0.1M Tris buffer, pH 7.4 recipe above (1000 ml)

0.5g Calcium Acetate

5.0g Zinc Acetate

5.0g Zinc Chloride

Mix to dissolve. The final pH will be approximately 6.5-7.0. Do not readjust the pH, as this will cause the zinc to come out of solution.

3. Store Zinc Fixative at room temperature.

4. Fix tissues in Zinc Fixative by placing freshly dis-sected tissue pieces (no larger than 5 mm in size) in Zinc Fixative and allowing to sit 6 hours at room temperature. After fixation, dehydrate tissues for one houreach in 70% ethanol, 90% ethanol, 95% ethanol, and 100% ethanol (2 changes) at room temperature. Tissues are then cleared in 2 changes of xylene for 50 minutes each at room temperature, followed by paraffin embedding.

Note: The use of an alkaline phosphatase detection system may be needed as this fixation method has been reported to be non-compatible with blocking of endogenous peroxidase activity.

REFERENCES

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Beckstead JH: Letter to the editor. Journal of Histochemistry and Cytochemistry 1995;43:345 (This is the actual formulation of the fixative).