

Preparation and use of Zamboni's-fixed Frozen Sections in Immunohistochemistry

ZAMBONI'S FIXATIVE (PARAFORMALDEHYDE/PICRIC ACID) SUGGESTED PREPARATION METHOD

1. Mix 20g paraformaldehyde with 150ml double-filtered, saturated aqueous picric acid.
 2. Heat to 60°C in fume cupboard.
 3. Add 2.52 per cent sodium hydroxide in water, drop by drop, until solution is clear.
 4. Filter solution and allow to cool.
 5. Make up to 1000ml with phosphate buffer.
3.31g NaH₂PO₄ · H₂O
33.77g Na₂HPO₄ · 7H₂O
1000ml distilled H₂O
- This fixative is stable at 25°C for 12 months.

PROCEDURES

1. Cut 7µm thick sections of frozen tissue and fix **immediately** in Zamboni's fixative for 10 minutes.
2. Wash 3 x 10 minutes in Tris buffered saline (pH7.6).
3. Cover with normal rabbit serum for 10 minutes.
4. Remove excess serum, cover with primary antibody and incubate for time indicated on data sheet at 4°C.
5. Wash in Tris buffered saline (pH7.6) for 2 x 5 minutes.
6. Cover with secondary antibody and incubate for 30 minutes at 25°C.
7. Wash in Tris buffered saline (pH7.6) for 2 x 5 minutes.
8. Cover with ABC reagent and incubate for 30 minutes at 25°C.
9. Wash in Tris buffered saline (pH7.6) for 2 x 5 minutes.
10. Develop with 3 3' diaminobenzidine tetrahydrochloride (DAB).
11. Counterstain.
12. Dehydrate, clear and mount sections.

REFERENCE

Stefanini M, De Martino C and Zamboni L. Fixation of ejaculated spermatozoa for electron microscopy. Nature. 216: 173-174 (1967).