**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 60oC 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 Na2H PO . 7H2O

1000ml distilled H2O

This fixative is stable at 25oC 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 4oC.

5. Wash in Tris buffered saline (pH7.6) for 2 x 5 minutes.

6. Cover with secondary antibody and incubate for 30 minutes at 25oC.

7. Wash in Tris buffered saline (pH7.6) for 2 x 5 minutes.

8. Cover with ABC reagent and incubate for 30 minutes at 25oC.

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).