Technical Data Sheet

Technovit H7100 / H8100

#14653 - 14654

Staining Protocols For Lymphoid Tissue

Normal immunostaining procedures for routine markers on sections obtained from lymphoid tissue.

Procedure

1. Dry the sections for two hours at 37°C on a slide warmer. This must be done whether the slides have been stored at 4°C for some time or just collected.
2. Pretreat the sections with trypsin at 37°C. Trypsin concentrations must be determined separately for each antibody. Use trypsin solution that has been preheated at 37°C for 30 minutes. Cover section with at least 100 micro liters of solution.
3. Wash in PBS for 10 minutes at room temperature. Refresh the buffer four to five times.
4. Preincubate in normal serum from the animal species in which the second antibody is raised for 30 minutes at 37°C. Only perform this step if aspecific background is present using a particular antibody.
5. Drip off excess serum and apply the first antibody in an appropriate concentration and incubate for two hours at 37°C.
6. Wash in PBS for 10 minutes at room temperature. Refresh the buffer four to five times.
7. Block endogenous preoxidase in a solution of 0.05% hydrogen peroxide in phosphate buffered saline, pH 7.4, for 30 minutes at room temperature.
8. Wash in PBS for 10 minutes at room temperature. Refresh the buffer four to five times.
9. Incubate in appropriate dilutions of the secondary antibody, containing 5% normal serum for 60 minutes at room temperature.
10. Wash in PBS for 10 minutes at room temperature. Refresh the buffer four to five times.
11. Develop the peroxidase activity in daminobenzidine (DAB).
12. Counterstain the sections in Hematoxylin or if a more advanced morphological detail is necessary, in periodic-acid-Schiff reagent.
13. Cover with Glycerin-gelatin and cover glass.