Protocol for Immunohistochemistry on Paraffin Sections

Deparaffinizing and Rehydration

1. Deparaffinize sections in 2 baths of xylene for 10 min each.
2. Rehydrate sections by sequentially incubation with 100%, 95%, 80% and 60% ethanol 5 min for each bath.
3. Rinse sections with distilled water 3 times for 3 min each.

Antigen Retrieval (Optional; without this step in our test)

4. Transfer sections into a container and cover with enough Tris-EDTA buffer (pH9) or Citrate buffer (pH6).
5. Heat in a microwave at the medium power for 10 min.
6. Allow slides to cool in the buffer for approx 35 min.

Incubation with Primary Antibodies

7. Rinse slides 3 times with 1X TBS for 3 min each.
8. Incubate slides with 3% H2O2 solution (diluted in distilled water) for 10 min to quench endogenous peroxidase activity.
9. Rinse slides 3 times with 1X TBS for 3 min each.
10. Prepare 5% normal blocking serum in 1X TBS, serum should be derived to the same species in which the secondary antibody is raised. Block the sections at room temperature for 1 h. (Take 5% BSA in 1X TBS instead if the corresponding serum is not available.)
11. Incubate sections with the antibody 10782-2-AP (Dilution: 1:3000; paraffin-embedded human brain (FTLD-U) tissue tissue slide) in 1X TBS at room temperature for 1.5 h or at 4°C overnight. Optimal dilution should be determined in pretests. Set up a negative control in parallel without applying primary antibody.
12. Rinse slides 3 times with 1X TBS for 3 min each.

Signal Detection (Reagents in EnVision Kits from Dako Company are applied)

13. Apply enough **Peroxidase Labeled Polymer** and incubate for 30 min at room temperature.
14. Rinse slides 3 times with **1X TBS** for 3 min each.
15. Prepare appropriate volume of substrate solution prior to use by mixing one drop of **Liquid DAB plus Chromogen** with 1 ml of **Substrate buffer** immediately. Apply the substrate carefully and incubate for 5-10 min till desired brown color is developed.
16. Rinse sections gently with enough distilled water.

Hematoxylin Counterstaining (Optional)

17. Immerse slides in a bath of hematoxylin to stain the nuclei for 3 min.
18. Rinse slides gently with distilled water bath.
19. Transfer slides into a solution containing 1% HCl and 99% ethanol for 10 s, and then into distilled water immediately.

Dehydration and Mounting

20. Immerse slides sequentially into 60%, 80%, 90% and 100% ethanol bath for 5 min each.
21. Get slides through two times of xylene bath for 5 min each.
22. Mount the section with a small drop of neutral balsam and coverslip. Air-dry in the hood.

Solutions

Citrate buffer	**1000 ml**	**1X TBS**	**1000 ml**
10 mM Tri-sodium citrate·$2H_2O$	2.9 g	20 mM Tris-base	2.4 g
1.9 mM Citric acid·H_2O	0.4 g	150 mM NaCl	8.7 g
Adjust pH to 6.0		Adjust pH to 7.6	
Add ddH2O to 1000 ml		Add ddH2O to 1000ml	

Tris-EDTA Buffer	**(10mM Tris Base, 1mM EDTA Solution, pH 9.0)**
Tris Base	1.21 g
EDTA	0.37 g
Distilled water	1000 ml