## PRODUCT-SPECIFIC PROTOCOLS

### IHC (10782-2-AP)

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Sample preparation</th>
<th>Antigen Retrieval</th>
<th>Blocking buffer</th>
<th>Primary antibody dilution</th>
<th>Incubation time</th>
<th>Signal detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>mouse brain tissue</td>
<td>Paraffin-embedded</td>
<td>Tris-EDTA buffer(pH9)</td>
<td>5% goat serum in TBS</td>
<td>1:2000</td>
<td>1.5 h at room temp</td>
<td>Dako/Agilent Envision kit</td>
</tr>
<tr>
<td>mouse brain tissue</td>
<td>Paraffin-embedded</td>
<td>None</td>
<td>5% goat serum in TBS</td>
<td>1:200</td>
<td>1.5 h at room temp</td>
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<tr>
<td>rat brain tissue</td>
<td>Paraffin-embedded</td>
<td>None</td>
<td>5% goat serum in TBS</td>
<td>1:200</td>
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</tr>
</tbody>
</table>
PROTOCOL (more details available upon request):

1. Deparaffinize sections in 2 baths of xylene for 10 min each.
2. Rehydrate sections by sequential 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 (check table above)
4. Transfer sections to a container and cover with antigen retrieval solution according to the table above.
5. Heat slides in a microwave on medium power for 10 min.
6. Allow slides to cool in the buffer for 35 min.
   Incubation with Primary Antibody and Signal Detection
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. Block the sections at room temperature for 1 h in Blocking buffer.
11. Incubate sections with primary antibody in 1X TBS
12. Rinse slides 3 times with 1X TBS for 3 min each.
13. Perform signal detection protocol according to the manufacturer's instructions.
14. Immerse slides in a bath of hematoxylin to stain the nuclei for 3 min.
15. Rinse slides gently in a distilled water bath.
16. Transfer slides to a solution containing 1% HCl and 99% ethanol for 10 sec, and then into distilled water immediately.
17. Immerse slides sequentially into 60%, 80%, 90%, and 100% ethanol bath for 5 min each.
18. Incubate slides in xylene bath for 5 min, remove, then incubate again for 5 min.
19. Mount the section with a small drop of neutral balsam and add a coverslip.
   Air-dry slides in the hood.
20. Examine slides under a microscope.

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