proteintech®
Antibodies | ELISA kits | Proteins

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# PRODUCT-SPECIFIC PROTOCOLS

## IHC (21756-1-AP)

| Sample type | Sample preparation | Antigen Retrieval | Blocking buffer | Primary antibody dilution | Incubation time | Signal detection |
|---|---|---|---|---|---|---|
| mouse eye tissue | Paraffin-embedded | Tris-EDTA buffer (pH 9.0) | 3% BSA in TBS | 1:200 | 1.5 h at room temp | Reagents for color development |

# PROTOCOL

**Deparaffinize and rehydrate**

1. Deparaffinize slides in 2 baths of xylene for 20 min each.
2. Rehydrate slides by sequential incubation with 100%, 95%, 80%, and 60% ethanol, 5 min for each bath.
3. Immerse slides with distilled water 3 times for 1 min each.

**Antigen Retrieval (check table above)**

1. Transfer slides to a container and cover with antigen retrieval solution according to the table above.
2. Heat slides in a microwave on medium power for 10 min.
3. Allow slides to cool in the buffer for 35 min.

**Incubation with Primary Antibody and Signal Detection**

1. Rinse slides 3 times with 1X TBS for 1 min each.
2. Incubate slides with 3% H2O2 solution (diluted in distilled water) for 10 min to quench endogenous peroxidase activity at room temperature.
3. Rinse slides 3 times with 1XTBS for 1 min each.
4. Block the slides at room temperature for 1 h in Blocking buffer.
5. Incubate slides with primary antibody (diluted in dilution buffer) for 1.5 h at room temperature.
6. Rinse slides 3 times with 1X TBST for 1 min each.
7. Incubate slides with sufficient peroxidase labeled polymer secondary antibody for 30 min at room temperature.
8. Rinse slides 3 times with 1X TBS for 1 min each.
9. Incubate slides with enough DAB for 2-5 min at room temperature till a brown color develops.
10. Rinse slides 3 times with distilled water for 1 min each.
11. Incubate slides in a bath of hematoxylin at room temperature to stain the nuclei for 3 min.
12. Rinse slides 3 times with 1X TBS for 1 min each.
13. Immerse slides in distilled water for 5 min.
14. Immerse slides sequentially into 60%, 80%, 90%, and 100% ethanol bath for 5 min each.
15. Immerse slides in xylene bath for 5 min, then immerse in another fresh xylene for 5 min.
16. Mount the slides with a small drop of neutral balsam and add a coverslip. Air-dry slides in the hood.
17. Examine slides under a microscope.

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