proteintech®
Antibodies | ELISA kits | Proteins

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# PRODUCT-SPECIFIC PROTOCOLS

## IF (25251-1-AP)

| Sample type | Sample preparation | Antigen retrieval | Blocking buffer | Antibody dilution buffer | Primary antibody dilution | Incubation time |
|---|---|---|---|---|---|---|
| mouse eye tissue | 4% PFA in 1×PBS, 10 min at room temp | Tris-EDTA (pH 9) | 5% goat serum in 1×PBS | 1% BSA in 1×PBS | 1:200 | 1.5 h at room temp |
| | Secondary antibody | | Secondary antibody dilution | Incubation time | Mounting Media | |
| | Multi-rAb CoraLite ® Plus 488-Goat Anti-Rabbit Recombinant Secondary Antibody (H+L) (RGAR002) | | - | 1.5 h at room temp | Hydromount? mounting media with DAPI | |

# 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 1XTBS for 5 min each.
2. Block the sections at room temperature for 1 hour in Blocking buffer.
3. Incubate sections with primary antibody in 1XTBS for 2 hours at room temperature or overnight at 4°C.
4. Rinse slides 3 times with 1XTBS for 5 min each.
5. Incubate sections with secondary antibody in 1XTBS for 1 hour at room temperature, protected from light.
6. Rinse slides 3 times with 1XTBS for 5 min each.
7. Mount the sections using an appropriate mounting media.
8. Examine slides under a fluorescent microscope.

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