proteintech®
Antibodies | ELISA kits | Proteins

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PRODUCT-SPECIFIC PROTOCOLS

IF (10993-1-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	
	CoraLite®488-Conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) (SA00013-2)		-	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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