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# OPEN ACCESS



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Protocol status: Working We use this protocol and it's working

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## Immunohistochemistry Protocol for Paraffin-Embedded Sections - General

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#### **ABSTRACT**

This protocol details general immunohistochemistry procedure.

**ATTACHMENTS** 

gegpbqbhp.pdf

#### **MATERIALS**

#### For DAB:

Solution-based: Sigma Cat# D5637; prepared in Tris buffer as per manufacturer's

instructions

SIGMAFAST DAB Tablets: Sigma Cat# 4293

SIGMAFAST DAB with Metal Enhancer - SIgma Cat# 0426

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## **PROTOCOL** integer ID:

62744

## **Keywords:**

Immunohistochemistry Protocol, Antigen retrieval



- 1 Dip slides in 100% Citrisolv 3 x 5 mins.
- 1.1 Dip slides in 100% Citrisolv 3 x (5) 00:05:00 (1/3).
- 1.2 Dip slides in 100% Citrisolv 3 x (5) 00:05:00 (2/3).
- **1.3** Dip slides in 100% Citrisolv 3 x (5) 00:05:00 (3/3).

## **Hydration**

- 2 Dip in 100% EtOH 2 x 2 mins.
- **2.1** Dip in 100% EtOH 2 x (5) 00:02:00 (1/2).

5m

5m

2.2 Dip in 100% EtOH 2 x 00:02:00 (2/2).

2m

- **3** Dip in 95% EtOH 2 x 2 mins.
  - 3.1 Dip in 95% EtOH 2 x 🕙 00:02:00 (1/2).

2m

3.2 Dip in 95% EtOH 2 x 00:02:00 (2/2).

2m

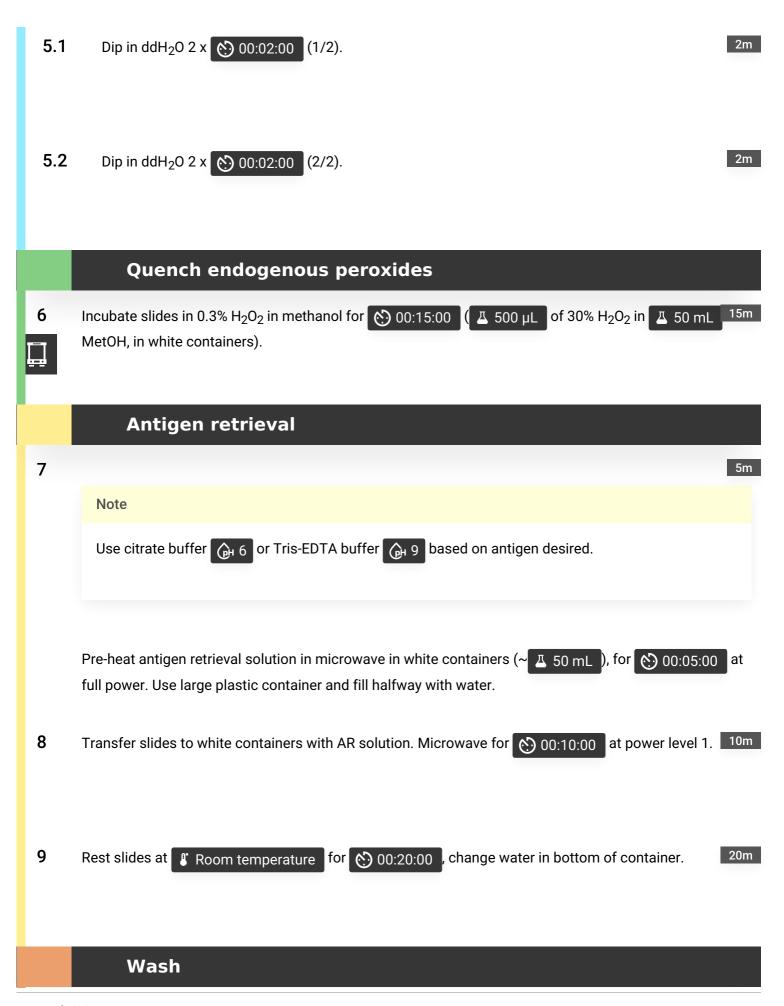
- 4 Dip in 80% EtOH 2 x 2 mins.
- 4.1 Dip in 80% EtOH 2 x (1/2).

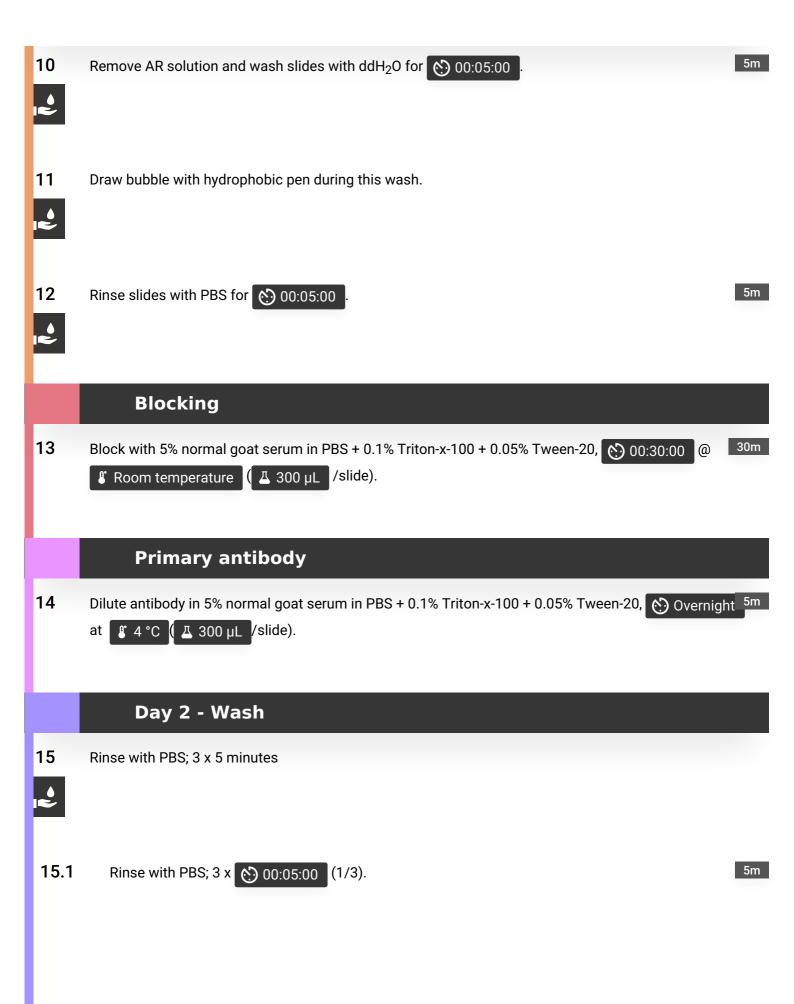
2m

4.2 Dip in 80% EtOH 2 x 00:02:00 (2/2).

2m

**5** Dip in ddH20 2 x 2 mins.





15.2 Rinse with PBS; 3 x 00:05:00 (2/3). 15.3 5m Rinse with PBS; 3 x (5) 00:05:00 (3/3).**Secondary antibody** 16 Add secondary antibody diluted 1:225 in PBS + 0.1% triton-X + 0.05% tween-20 with 5% normal goat serum ( $\sim$   $\pm$  300  $\mu$ L /slide). 16.1 Commonly: biotinylated anti-rabbit IgG (H+L) made in goat (Vector Labs, BA-1000) or biotinylated antimouse IgG (H+L) made in goat (Vector Labs, BA-9200). 17 Incubate at Room temperature for 01:00:00 3h 02:00:00 Wash 18 Rinse with PBS; 3 x 5 minutes. 18.1 5m Rinse slides with PBS; 3 x (5) 00:05:00 (1/3).

Rinse slides with PBS; 3 x (2/3).

18.2

**18.3** Rinse slides with PBS; 3 x (5) 00:05:00 (3/3).

5m

# **Amplification**

Prepare amplification solution at least 000:30:00 before use.

30m

- 20 Amplification with VECTASTAIN® Elite® ABC HRP Kit.
- 20.2 Incubate at Room temperature for 01:00:00 02:00:00

3h

## Wash

- 21 Rinse slides with PBS; 3 x 5 minutes
- 21.1 Rinse slides with PBS; 3 x (5) 00:05:00 (1/3).

21.2 Rinse slides with PBS; 3 x (5) 00:05:00 (2/3).

5m

21.3 Rinse slides with PBS; 3 x (5) 00:05:00 (3/3).

5m

## **Substrate development**

22

Note

Use DAB or eDAB depending on antigen being detected.

For regular DAB:

22.1 Add  $\bot$  1 mL of DAB to  $\bot$  150 mL of [M] 50 millimolar (mM) Tris +  $\bot$  50  $\mu$ L 30% H<sub>2</sub>0<sub>2</sub>.



22.2 Dip slides into DAB solution, develop until colour appears ~ (5) 00:03:00

- **23** For eDAB (SIGMAFAST™ DAB with Metal Enhancer):
- Add 1 tablet DAB/Cobalt and 1 tablet Urea Hydrogen Peroxide to 5 mL ddH<sub>2</sub>O, vortex until dissolved.

Note

Prepare immediately before use.

23.2 Use ~ Δ 300 μL /slide, develop until colour appears ~ 👏 00:03:00

3m



**24** Rinse with PBS to stop reaction.





Rinse with  $ddH_20$  for  $\bigcirc$  00:05:00

5m



## Counterstain

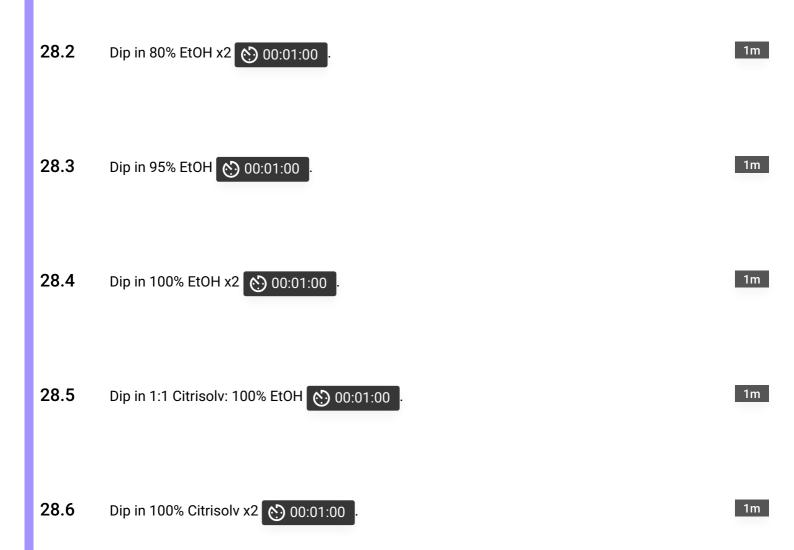
26 Counterstain with 1:2 Hematoxylin:ddH<sub>2</sub>O, dip slides in quickly.

27 Remove excess hematoxylin in ddH<sub>2</sub>O; 2X.

## **Dehydration**

5 dips, 1 minute each:

**28.1** Dip in 50% EtOH 00:01:00



## Coverslip

29 Coverslip with permount solution, allow slides to dry before viewing on microscope.