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Fluorescent immunochemistry

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Works for me

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ABSTRACT

General fluorescent immunostaining protocol for paraffin sections

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PROTOCOL INTEGER ID

46453

Deparaffinizing

25m

24m

- 1 - [Citrisolv](#) Contributed by users ⌚ 00:05:00
- [Citrisolv](#) Contributed by users ⌚ 00:10:00
- Ethanol 100° ⌚ 00:03:00
- Ethanol 95° ⌚ 00:03:00
- Ethanol 75° ⌚ 00:03:00

Antigen Retrieval

12m

45m

- 2 Microwave for 3 x ⌚ 00:04:00 with microwaving solution (kept @ 4 °C)
 Let it cool ⌚ On ice until ⌚ Room temperature

- 2.1 Microwaving solution:
 9 mL Citric Acid 0.01 M
 41 mL Sodium Citrate 0.01 M

450 mL H₂O

Permeabilization 20m

- 3 Permeabilization with TBS 1x; **0.1 % (v/v)** Triton 100 (20 min @ **Room temperature**) 20m

Blocking 30m

- 4 Blocking with the Blocking Solution **00:30:00** 30m

Primary antibody 4m

- 5 Anti Insulin (Guinea Pig) 1/800
Anti Ki67 (Rabbit) 1/200
(**Overnight** @ **4 °C**) 4m

Wash 15m

- 6 Wash with TBS 1 x; 0.1% Tween, rocking (3 x **00:05:00**) 15m

Secondary Antibody 2h

- 7 Anti Guinea Pig-FITC 1/800
Anti Rabbit-Cy3 1/200
(**02:00:00 minimum** @ **Room temperature**) 2h

Wash 5m

- 8 Wash with TBS 1 x; 0.1% Tween, rocking (3 x **00:05:00**) 5m

Final wash 5m

- 9 Wash with TBS 1 x rocking (**00:05:00**) 5m

Mount

- 10 Coverslip with antifading agent mounting media with or without DAPI
Use Anti-fading Mounting Media
Step 10 includes a Step case.