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# 🌐 Immunohistochemistry for FOXP3+ staining in Breast Cancer Tissue

Freda Halim<sup>1</sup>

<sup>1</sup>Faculty of Medicine, Pelita Harapan University, Tangerang



Freda Halim

Faculty of Medicine, Pelita Harapan University, Tangerang

## ABSTRACT

These are protocols used for study of FOXP3+ Cell Count in Luminal B Her-2 negative BC patients. We used using paraffin sections of 66 samples and stained the slides for FOXP3+ antibody, using primary antibody clone 236A/E7 Abcam Cambridge UK dilution 1:50

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

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 82571

## Deparaffinization and Rehydration

23m

- 1 Incubate slides in Xylenes for 3 minutes 3m
- 2 Incubate slides in Xylenes 3m
- 3 Incubate slides in Xylenes 3m
- 4 Rehydrate slides in 100% Ethanol 3m
- 5 Rehydrate slides in 96% Ethanol 3m
- 6 Rehydrate slides in 70% Ethanol 3m
- 7 Rinse with running water and aquadest 5m

## Blockage of Endogenous Peroxidase

20m

- 8 Incubate slides in 3% H2O2 15m

9 Rinse slides with running tap water and aquadest

5m

## Antigen Retrieval

1h

10 Antigen Retrieval with Tris EDTA ( pH9) with pressure cooker, in 950 Celcius temperature

20m

11 Open the lid and cool down in room temperature

15m

12 Rinse slides with running water and aquadest

5m

13 Rinse in PBS ( Phosphate Buffer Saline ) in pH 7.40-7.60

5m

14 Excell Block

10m

15 Rinse in PBS in pH 7.40-7.60

5m

## Primary Antibody

1h 5m

16 Wipe excess liquid around the tissue

**17** apply primary antibody ( Clone 236A/E7 Abcam Cambridge UK dilution 1:50 ) 120μL

**18** Incubate for 60 minutes

1h

**19** Rinse with PBS

5m

## Secondary Antibody

40m

**20** Apply Excell Link as secondary antibody

15m

**21** Rinse with PBS

5m

**22** Apply Excell HRP as secondary antibody

20m

## Signal Detection/Histochemistry

25m

**23** Apply DAB ( Diamino-benzidine ) 80-100μL for 10 minutes , Rinse with running tap water and aquadest for 5 minutes

15m

**24** Apply Hematoxyline for 1 minutes, Rinse with running tap water and aquadest for 5 minutes

5m

**25** Apply Tatcha's bluing solution and rinse with running tap water and aquadest for 5 minutes

5m

## Dehydration and Clearing

20m

**26** Clear excess water from the slides

**27** Dehydrate Slides in 70%, 96%, 100% for 5 minutes each

15m

**28** Incubate slides in Xylenes for 5 minutes

5m

**29** Mount the slides