

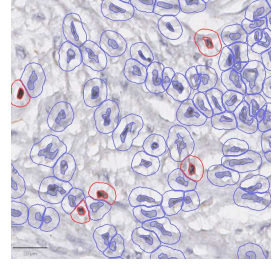


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## Immunohistochemistry for FOXP3+ staining in Breast Cancer Tissue

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**Protocol status:** Working

**We use this protocol and it's working**

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**Keywords:** immunohistochemistry for foxp3, study of foxp3, breast cancer tissue, using primary antibody, immunohistochemistry, slides for foxp3, foxp3, primary antibody, antibody, cell count

## 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

## Troubleshooting



## Deparaffinization and Rehydration

23m

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

3m

3m

3m

3m

3m

3m

5m

## Blockage of Endogenous Peroxidase

20m

- 8 Incubate slides in 3% H2O2
- 9 Rinse slides with running tap water and aquadest

15m

5m

## Antigen Retrieval

1h

- 10 Antigen Retrieval with Tris EDTA ( pH9) with pressure cooker, in 950 Celcius temperature
- 11 Open the lid and cool down in room temperature

20m

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 Hematoxylline 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

## Protocol references

Asano Y, Kashiwagi S, Goto W, Kurata K, Noda S, Takashima T, et al. Tumour-infiltrating CD8 to FOXP3 lymphocyte ratio in predicting treatment responses to neoadjuvant chemotherapy of aggressive breast cancer. Br J Surg. 2016;103(7):845–54.