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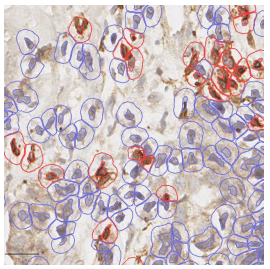
Immunohistochemistry for CD8+ staining in Breast Cancer Tissue



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Freda Halim[1]

[1]Faculty of Medicine, Pelita Harapan University, Tangerang

Freda Halim
Faculty of Medicine, Pelita Harapan University, Tangerang

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

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Abstract

These are protocols used for study of CD8+ Cell Count in Luminal B Her-2 negative BC patients. We used using paraffin sections of 66 samples and stained the slides for CD8+ antibody, using primary antibody CD8 (clone C8/144B, dilution 1:100; Dako

Deparaffinization and rehydration 23m

1 Incubate slides in Xylenes for 3 minutes 9m

2 Rehydrate slides in 100% Ethanol, 96% Ethanol, 70% Ethanol each for 3 minutes 9m

3 Rinse with running tap water and aquadest 5m

Blockage of Endogenous Peroxidase 20m

4 Incubate slides in 3% H202 for 15 minutes 15m

5 Rinse with running tap water and aquadest 5m

Antigen Retrieval 1h

6 Antigen Retrieval with Tris EDTA (pH9) with pressure cooker, in 95 degree Celcius temperature 20m

7 Open the lid and cool down in room temperature 15m

8 Rinse slides with running water and aquadest for 5 minutes 5m

9 Rinse in PBS (Phosphate Buffer Saline) in pH 7.40-7.60 5m

10 Excell Block 10m

11 Rinse in PBS in pH 7.40-7.60 5m

Primary Antibody

1h 5m

12 Wipe excess liquid around the tissue, apply primary antibody :CD8 (clone C8/144B, dilution 1:100; Dako

13 Incubate for 60 minutes and then rinse with PBS for 5 minutes 1h 5m

Secondary Antibody

40m

14 Apply Excell Link as secondary antibody for 15 minutes, then rinse with PBS for 5 minutes 20m

15 Apply Excell HRP as secondary antibody 20m

Signal Detection/Histochemistry

27m

16 Apply DAB (Diamino-benzidine) 80-100µL for 10 minutes , Rinse with running tap water and aquadest for 5 minutes 15m

17 Apply Hematoxylline for 1 minutes, Rinse with running tap water and aquadest for 5 minutes 6m

18 Apply Tatcha's bluing solution and rinse with running tap water and aquadest for 5 minutes 6m

Dehydration and Clearing

20m

19 Clear excess water from slides and Dehydrate Slides in 70%, 96%, 100% for 5 minutes each 15m

20 Incubate slides in Xylenes for 5 minutes 5m

21 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.