Immunohistochemistry for CD8+ staining in Breast Cancer Tissue

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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

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

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**Deparaffinization and rehydration**

1. Incubate slides in Xylenes for 3 minutes

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

3. Rinse with running tap water and aquadest

**Blockage of Endogenous Peroxidase**

4. Incubate slides in 3% H2O2 for 15 minutes

5. Rinse with running tap water and aquadest

**Antigen Retrieval**

6. Antigen Retrieval with Tris EDTA (pH9) with pressure cooker, in 95 degree Celcius temperature
7. Open the lid and cool down in room temperature

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

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

10. Excell Block

11. Rinse in PBS in pH 7.40-7.60

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

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

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

17. Apply Hematoxylline for 1 minute, Rinse with running tap water and aquadest for 5 minutes

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

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

20. Incubate slides in Xylenes for 5 minutes

21. Mount the slides