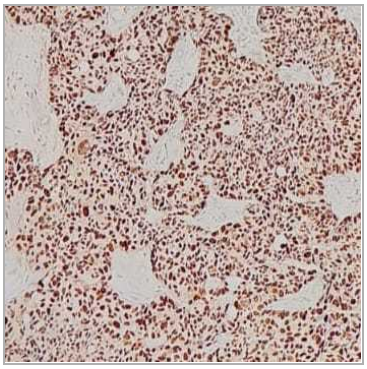

MAR 20, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.5qpvord7dv4o/v1

Protocol Citation: Freda Halim 2023. Immunohistochemistry for p53 staining in Breast Cancer Tissue. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5qpvord7dv4o/v1

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

Created: Mar 13, 2023

Last Modified: Mar 20, 2023

PROTOCOL integer ID:
78640

Immunohistochemistry for p53 staining in Breast Cancer Tissue

Freda Halim[1,2]

[1]Doctoral Degree, Faculty of Medicine, Universitas Padjajaran;
[2]Surgery Department, Faculty of Medicine, Pelita Harapan Unviersity

Freda Halim

ABSTRACT

These are protocols used for study of p53 expression differences in Luminal B Her-2 negative patients. The aim of the study is to show p53 expression differences in Luminal B Her-2 negative patients with and without primary endocrine therapy resistance.We used using paraffin sections of 67 samples and stained the slides for p53 expression, using primary antibody Clone DO-7 (DAKO).

Deparaffinization and Rehydration

1 Incubate slides in Xylenes for 3 minutes 3m

2 Incubate slides in Xylenes for 3 minutes 3m

3 Incubate slides in Xylenes for 3 minutes 3m

4 Rehydrate slides in 100% Ethanol for 3 minutes 3m

5 Rehydrate slides in 96% Ethanol for 3 minutes 3m

6 Rehydrate slides in 70% Ethanol for 3 minutes 3m

7 Rinse with running tap water and aquadest for 5 minutes 5m

Blockage of Endogenous Peroxidase

8 Incubate slides in 3% H_20_2 for 15 minutes 15m

9 Rinse slides with running tap water and aquadest for 5 minutes 5m

Antigen Retrieval

40m

10 Antigen Retrieval

10.1 Antigen Retrieval with Tris EDTA (pH9) with pressure cooker, in 95^0 Celcius temperature — 20m

10.2 Open the lid and cool down in room temperature — 15m

10.3 Rinse slides with running water and aquadest for 5 minutes — 5m

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

10.5 Excell Block — 10m

10.6 Rinse in PBS in pH 7.40-7.60 — 5m

Primary Antibody

40m

11 Wipe excess liquid around the tissue

12 apply primary antibody (clone DO7, Dako) 120µL

13 Incubate for 60 minutes 1h

14 Rinse with PBS 5m

Secondary Antibody

40m

15 Apply Excell Link as secondary antibody 15m

16 Rinse with PBS 5m

17 Apply Excell HRP as secondary antibody 20m

Signal Detection/ Histochemistry

27m

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

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

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

Dehydration and Clearing 20m

21 Clear excess water from the slides

22 Dehydrate Slides in 70%, 96%, 100% for 5 minutes each 15m

23 Incubate slides in Xylenes for 5 minutes 5m

24 Mount the slides