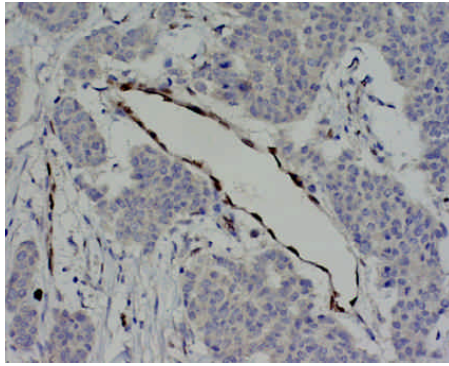


Incubated 2hrs at RT
(Endothelial cells nuclear staining on breast cancer tissue sections)



Incubated O/N at 4 °C

BioCheck, Inc.
323 Vintage Park Drive
Foster City, CA 94404
Telephone (650) 573-1968
Facsimile (650) 573-1969

IHC Protocol for Rabbit Monoclonal anti-Mouse/Human Id1
(Cat #BCH-1/#195-14-cDNA)

Intended use: For research use only

Recommend Kit: Dako Cytomation EnVision™+ Kits (Dako Catalog # K4010 and K4011)

Antibody Concentration: 0.05 mg/ml

Suggested Dilution: 1:100

Protocol:

1. Deparaffinize and rehydrate the tissue sections.
2. Immerse slides in 3 % H₂O₂ in Methanol for 10 minutes.(Optional).
3. Wash with distilled water 3 times with 2 minutes each.
4. Pretreat slides with Heat induced epitope retrieval (HIER) using **50 mM Tris Buffer containing 1 mM EDTA (pH 8.0)**.
5. Cool down the slides in the buffer at room temperature for 15-20 minutes after HIER.
6. Apply rabbit monoclonal anti-mouse/human Id1 (Cat# BCH-1/#195-14; 0.05 mg/ml) at **1:100 dilution**, and incubate for **2 hours at room temperature or overnight at 4 °C**.
7. Wash with PBS (10 mM, pH 7.4) 3 times, with 2 minutes each.
8. Apply EnVision™+ (Dako K4010 or K4011) PHRP-Gt x Rb IgG (ready-to-use form), and incubate for 30 minutes.
9. Wash with PBS (10 mM, pH 7.4) 3 times, with 2 minutes each.
10. Apply DAB solution **for 5 minutes**.
11. Wash with tap water thoroughly.
12. Counterstained with hematoxylin, wash with tap water again, and then mounted as regular procedure.

*** Please Note:**

- The dilution factor of rabbit monoclonal anti-m/h Id1 is critical. The optimal conditions need to be adjusted by each lab.
- Counterstain step needs to be light since the specific nuclear staining may be weak.