Sentinel Lymph Node Rapid IHC Kit
For Rapid Immunohistostaining of Sentinel Lymph Node
Cat. No. 28-8700

INTENDED USE
For Research Use Only, Not for Use in Diagnostic Procedures.

Sentinel Lymph Node RIHC Kit is a sensitive and rapid immunohistochemistry (RIHC) kit that is specific for detection of cytokeratins. The Kit may be useful when conducting research studies in metastasized breast carcinoma in frozen sections of sentinel lymph nodes. The kit employs a cocktail of monoclonal pan-cytokeratin antibodies directly conjugated to horseradish peroxidase (HRP) using Invitrogen’s proprietary HRP Polymer Technology. The pan-cytokeratin antibodies react with seven different human cytokeratin isoforms that are specifically expressed in squamous cell carcinoma, adenocarcinoma, and transitional cell carcinoma. The entire procedure of the test, including handling time, takes less than 8 minutes to complete.

INTRODUCTION
Axillary lymph node (ALN) dissection is an essential component for management of breast cancer. The risk of ALN metastasis is directly related to the size of the primary tumor. Therefore, ALN dissection performed in early breast cancer patients, with small tumors found at a high frequency, often reveals healthy lymphatic nodes. In the absence of accurate procedures to evaluate the state of ALNs, complete ALN dissection has remained an integral part of standard breast surgery. However, the removal of all ALNs deprives the patient of lymphatic tissue, thereby increasing susceptibility to infections, and reducing in some cases arm mobility and skin sensitivity, in addition to other side effects such as pain and/or lymphoedema.

Sentinel lymph node (SLN) biopsy is a safe and accurate staging procedure for breast carcinoma in patients without clinical evidence of ALN metastasis. The technique of SLN biopsy represents a minimally invasive diagnostic procedure that is able to predict the histological state of the ALN through the examination of the first axillary node to receive lymphatic drainage from a primary breast carcinoma. SLN biopsy has the potential of avoiding ALN dissections in a large percentage of patient. Previous studies have indicated that only 1% of ALN dissections will be positive for metastatic carcinoma in cases in which the SLN biopsy was negative for metastatic carcinoma by morphology and immunohistochemistry. Furthermore, SLN biopsies positive for metastatic carcinoma predict ALN metastasis in 30% to 70% of cases. Therefore, SLN biopsy can be used to determine which patients will benefit from ALN dissections.

Intraoperative SLN frozen sections (FSs) or touch preparations (TPs) have been routinely examined for the detection of nodal metastasis, thus allowing for immediate ALN dissection and avoiding the need for reoperation. Various techniques including immunohistochemistry, reverse transcription polymerase chain reaction and flow cytometry have been employed in the histopathological evaluations of SLN biopsies, in addition to routine hematoxylin and eosin staining.

Rapid immunohistochemistry (RIHC) is a technique that has been used to aid in the intraoperative diagnosis of several malignancies, including gastric carcinoma, pancreatic carcinoma, and malignant melanoma. However, intraoperative RIHC on FSs and TPs to detect metastatic breast carcinoma has so far yielded mixed results. Potential problems involved include low sensitivity (increase in false-negatives) and long operating time (delay in results).

Sentinel Lymph Node RIHC Kit is highly sensitive for the detection of cytokeratins. It is useful when researching metastasized breast carcinoma in frozen sections of sentinel lymph nodes. This standardized and complete kit employs a cocktail of anti-pan-cytokeratin monoclonal antibodies directly conjugated to horseradish peroxidase (HRP) using Invitrogen’s proprietary HRP Polymer Technology. Cytokeratins are specific to epithelial cells throughout differentiation and, therefore, can be used as markers for detection of metastatic carcinomas with epithelial origins. Sentinel Lymph Node RIHC Kit has a total test-turnaround time of less than 8 minutes, and is simple and easy to use, with all reagents provided in convenient dropper bottles.

For Export Use Only
REAGENTS AND MATERIALS PROVIDED
(Good for 20 Tests/Slides)
A. 1 dropper bottle (3 mL) of Ready-To-Use HRP polymer-conjugated anti-pan cytokeratin antibodies
B1. 1 dropper bottle (1 mL) of 20X substrate buffer
B2. 1 dropper bottle (1 mL) of 20X DAB chromogen
B3. 1 dropper bottle (1 mL) of 20X hydrogen peroxide
1 package insert with the test and staining protocol

NOTE: DAB substrate solution should be made fresh during each assay by adding one drop each of B1, B2, and B3 into 1 mL of distilled water.

REAGENTS & MATERIALS REQUIRED BUT NOT PROVIDED
• Acetone
• Mayer’s hematoxylin
• Tris-buffered saline (TBS; 50 mM Tris, pH 7.8, 150 mM NaCl) or PBS
• Distilled or deionized water
• Mounting media, such as Clearmount™ (Cat. No. 00-8010) or Histomount™ (Cat. No. 00-8030)
• Cover slips
• Xylene
• Glass jars
• Humidified chamber at 37 °C
• Pipettor
• Timer
• Light microscope

STORAGE
Store at 2-8 °C. Do not freeze the kit.

STABILITY
The kit is stable for at least one year at 4 °C. Do not use after expiration date. Reagents should not be used if deterioration or substantial loss of activity is evident.

PRINCIPAL OF TEST
Cytokeratin antigens on metastatic breast cancer cells in frozen sections of the sentinel lymph nodes are specifically recognized and bound by anti-cytokeratin antibodies conjugated with HRP polymer. The DAB substrate solution is added to the samples to form the colored deposits in the presence of HRP polymer conjugated with the pan-cytokeratin antibodies and complexed to the antigens.

STAINING PROCEDURE

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<thead>
<tr>
<th>Step</th>
<th>Description</th>
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<tbody>
<tr>
<td>Step 1. Frozen Tissue mount</td>
<td>Mount freshly dissected frozen tissue section (4-5 μm in thickness) on slide</td>
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| Step 2. Frozen Tissue fixation | Fix frozen tissue section immediately in acetone for 20 seconds
Rinse twice with TBS or PBS |
| Step 3. Antibody incubation | Apply 3-4 drops (100~150 μL) of anti-pan-cyokeratin/HRP conjugates
Incubate for 3 minutes at 37 °C
Rinse twice with TBS or PBS |
| Step 4. Substrate addition | Add 3-4 drops of DAB substrate solution
Incubate for 3 minutes at 37 °C
Rinse twice with water |
| Step 5. Counterstaining | Stain with Mayer’s hematoxylin for 10 seconds
Rinse twice with water
Evaluate the slide prior to permanent mounting |
| Step 6. Coverslip mount | Apply coverslip with mounting media |
NOTE: All reagents should be equilibrated to room temperature prior to immunostaining. While doing the anti-cytokeratin incubation, prepare the DAB substrate solution as previously instructed.
EXPECTED RESULTS
The pan-cytokeratin antibodies used in the kit react with human cytokeratin isoforms that are specific to epithelial cells throughout differentiation and can be used as markers for detection of metastatic carcinomas with epithelial origins such as squamous cell carcinoma, adenocarcinoma, and transitional cell carcinoma. 26-28. Although developed for detection of cytokeratins expressed in metastasized breast cancer cells in frozen sections of sentinel lymph nodes, the rapid IHC kit performed equivalently well on the paraffin embedded tissue sections. The staining intensity usually reflects the effects of tissue preparation as well as antigen concentration. An intense staining indicates a relatively higher concentration of cytokeratins, while a lighter staining is indicative of a lower cytokeratin concentration.

REFERENCE

TRADEMARKS
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