

## NB110-57284 Protocol

### Protocol specific for Lck Antibody (NB110-57284)

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**Immunohistochemistry Protocol for Paraffin-embedded Tissues**

**1. Solutions and reagents**

**1.1.** Xylene

**1.2.** Ethanol, anhydrous denatured, histological grade (100%, 95%, 70%)

**1.3.** Washing buffer: TBST washing buffer: 1XTBS/0.1% Tween-20

To prepare stock solution of 10X TBS: add 24.2 g Trizma base and 80 g sodium chloride to 1L of dH<sub>2</sub>O. Adjust pH to 7.6.

Working solution. 1XTBST/0.1% Tween-20: add 100ml 10XTBS to 900 ml dH<sub>2</sub>O. Add 1 ml Tween-20 and mix well.

**1.4.** Distilled water (dH<sub>2</sub>O)

**1.5.** Antigen Retrieval Solution: 0.01M Sodium Citrate Buffer, pH 6.0

To prepare stock solutions:

Solution A. 0.1 M citric acid solution: dissolve 21.0 g of citric acid, monohydrate (C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>.H<sub>2</sub>O) in 1 liter of dH<sub>2</sub>O.

Solution B. 0.1M sodium citrate solution: dissolve 29.4 g trisodium citrate dihydrate (C<sub>6</sub>H<sub>5</sub>Na<sub>3</sub>O<sub>7</sub>.2H<sub>2</sub>O) in 1 liter of dH<sub>2</sub>O.

Working solution: Add 9 ml of Stock solution A and 41 ml of stock solution B to 450 ml of dH<sub>2</sub>O. Adjust pH to 6.0.

**1.6.** 3% Hydrogene Peroxide

**1.7.** Blocking buffer: PBS (Dulbeccos Phosphate Buffered Salts, 1X, catalog #21-031-CV from Mediatech, Inc.) + 10% serum (serum origin depends on the host of the secondary antibody)

**1.8.** Hematoxylin QS (catalog #H-3404 from Vector Laboratories, Inc.)

**1.9.** Permanent Mounting medium (VectaMount, catalog# H-5000 Vector Laboratories, Inc.)

**2. Deparaffinization/Rehydration**

**2.1.** Heat slides in an oven at 65C for 1 hour.

**2.1.1.** Heat slides in an oven at 65C for 1 hour.

**2.1.2.** De-paraffinize/hydrate using the following series of washes: two Xylene washes (5 min each), followed by two 100% ethanol rinses (5 min each), followed by 95% ethanol, 70% ethanol, 50% ethanol, 30% ethanol, followed by H<sub>2</sub>O and a TBST wash for 5 min on a shaker.

**2.2.** Antigen Retrieval

**2.2.1.** Immerse slides into staining dish containing Antigen Retrieval Solution.

**2.2.2.** Place covered staining dish into the rice cooker. Add 120 ml of dH<sub>2</sub>O.

**2.2.3.** When cook is turned to warm (about 20 to 30 min), unplug the cooker and remove the staining dish to the bench top.

**2.2.4.** Allow to cool down, without cover, for 20 min.

**2.3.** Staining

**2.3.1.** Wash slides with TBST for 5 min on a shaker.

**2.3.2.** Inactivate endogenous peroxidase by covering tissue with 3% hydrogen peroxide for 10 min.

**2.3.3.** Wash slides three times with TBST (3 min each on a shaker).

**2.3.4.** Block slides with the blocking solution for 1 hour.

**2.3.5.** Dilute primary antibody in the blocking buffer per recommendation on the data sheet.

**2.3.6.** Apply primary antibody to each section and incubate overnight in the humidified chamber (4C).

**2.3.7.** Wash slides three times with TBST (3 min each on a shaker).

**2.3.8.** Apply to each section secondary HRP-conjugated anti-rabbit antibody diluted in the blocking solution per manufacturers recommendation; incubate for 1 hour at room temperature.

**2.3.9.** Wash slides three times with TBST (3 min each on a shaker).

**2.3.10.** Add freshly prepared DAB substrate to the sections.

**2.3.11.** Incubate tissue sections with the substrate at room temperature until suitable staining develops (generally 2 to 5 min).

**2.3.12.** Rinse sections with water.

**2.3.13.** Counterstain with Hematoxylin.

**2.3.14.** Rinse sections with water.

**2.3.15.** Dehydrate samples using two rinses with 100% Ethanol (20 dips per rinse) followed by two rinses with Xylene (30 dips per rinse).

**2.3.16.** Mount coverslips on slides using Permount medium.