Version 2b: Last updated 6 August 2025

ab16667

Protocol for Ki-67

Clone ID: SP6 Host Species: Rabbit monoclonal

This product is for research use only and is not intended for diagnostic use.

Protocol

1. Deparaffinization/Rehydration

1.1. Heat slides in an oven at 63 °C - 65 °C for about 1 hour.

1.2. Deparaffinize/hydrate using the following series of washes: twice Xylene rinses (5-10 min each), followed by twice anhydrous ethanol rinses (5 min each), twice 95% ethanol rinses (5 min each), 85% ethanol rinse (3 min), 75% ethanol rinse (3 min), and finally thrice distilled water washes.

2. Antigen Retrieval

This is recommended Heat Induced Epitope Retrieval (HIER) using Decloaking Chamber/Pressure Cooker.

2.1. Add 500 ml of distilled water to Decloaker/Pressure Cooker.

2.2. Immerse slides into staining dish containing Antigen Retrieval Solution: **Heat mediated antigen retrieval using ab93678 (Citrate buffer, pH 6.0)**. Place staining dish into Decloaking chamber.

2.3. Program to run for 30 s at 125 °C or 15-30 min at 110 °C.

2.4. Let it cool down to room temperature (10-30 min).

2.5. Removes slides and rinse in distilled water.

2.6. Proceed to Staining step.

3. Staining

3.1. Wash slides with distilled water thrice (3 min each if on a shaker).

3.2. Inactivate endogenous peroxidase by covering tissue with 3% hydrogen peroxide for 5-10 min.

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

3.4. Block slides with the blocking solution for 30-60 min.

3.5. Dilute primary antibody in primary antibody diluent per recommendation on datasheet.

3.6. Apply primary antibody to each section and **incubate overnight in the humidified chamber (2~8 ºC)**.

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

3.8. Apply to each section secondary HRP-conjugated anti-rabbit antibody diluted in the blocking solution per manufacturer's recommendation; incubate for 30 min at room temperature.

3.9. Wash slides three times with TBST (5 min each on a shaker).

3.10. Add freshly prepared DAB substrate to the sections and incubate until stain develops (10-60 s, according to the instruction).

3.11. Rinse sections with distilled water.

3.12. Counterstain with Hematoxylin (1-2 min, according to instruction).

3.13. Rinse sections with water.

3.14. Dehydrate samples using 75% ethanol rinse (3 min), 95% ethanol rinse (3 min), twice anhydrous ethanol rinses (3 min each), followed by twice rinses with Xylene or Histoclear (5 min each).

3.15. Dry slides and mount the coverslips onto slides using permanent mounting medium.

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