Protocol Booklet for ab236676

MATERIALS SUPPLIED

1 x Anti-PD-L1 antibody [28-8]	50 ul
1 x Universal HIER antigen retrieval reagent (10X)	100 ml
2 x Rabbit specific IHC polymer Amplifier	15 ml
2 x Rabbit specific IHC polymer Detector	15 ml

For more details, please via

ab205921
https://www.abcam.com/ps/products/205/ab205921/documents/ab205921%20IHC,%20WB%20&%20FC%20Protocol%20Booklet%20(clone%2028-8)v3%20(website).pdf

ab208572
https://www.abcam.com/ps/products/208/ab208572/documents/ab208572%20Antigen%20retrieval%20reagent%20(10X)v1%20(website).pdf

ab209101
https://www.abcam.com/ps/products/209/ab209101/documents/ab209101%20Rabbit%20specific%20IHC%20polymer%20detection%20kit%20HRPDAB%20v1a%20(website).pdf

IMMUNOHISTOCHEMISTRY PROTOCOL

1. Solutions and reagents

1.1. Xylene

1.2. Ethanol, anhydrous denatured, histological grade (100%, 95%, 70%, 50%)

1.3. Washing buffer/TBST: 1× TBS/0.1% Tween-20, pH to 7.6.

1.4. Distilled water (dH_2O)

1.5. Universal HIER antigen retrieval reagent (10X) (ab208572)

Dilute concentrated Universal HIER antigen retrieval reagent (10X) at a ratio of 1:10

(1mL Universal HIER antigen retrieval reagent (10X) to 9 ml of deionized water)

1.6. 3% Hydrogen Peroxide

1.7. Blocking Buffer: 10% serum in PBS (serum origin depends on the host of the secondary antibody)

1.8. Antibody Diluent (ab64211)

1.9. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101): Amplifier and Detector

1.10. DAB Substrate Kit (ab64238)

1.11. Hematoxylin

1.12. Permanent Mounting Medium

2. Protocol

2.1. Deparaffinization/Rehydration

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

2.1.2. Deparaffinize/hydrate using the following series of washes: two Xylene washes (3 min each), followed by two 100% ethanol rinses (3 min each), followed by 95% ethanol, 70% ethanol, 50% ethanol, 30% ethanol, followed by TBST wash for 3 min on a shaker.

2.2. Antigen Retrieval

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

2.2.1. Add 500 ml of dH_2O to Decloaker/Pressure Cooker.

2.2.2. Immerse slides into staining dish containing 1X Universal Antigen Retrieval Solution. Place staining dish into Decloaking chamber.

2.2.3. Program to run for 30 s at 125 °C.

2.2.4. Let it cool down to room temperature (10-20 min).

2.2.5. Removes slides and rinse in TBST.

2.2.6. Proceed to Staining step.

2.3. Staining

2.3.1. Wash slides with TBST for 3 min on a shaker.

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

2.3.5. Dilute primary antibody in antibody diluent to 2 μg/ml.

2.3.6. Apply primary antibody to each section and incubate overnight in the humidified chamber (2~8 ºC).
2.3.7. Wash slides three times with TBST (3 min each on a shaker).
2.3.8. Apply Amplifier to slides, and incubate for 10 min at RT.
2.3.9. Wash slides three times with TBST (3 min each on a shaker).
2.3.10. Apply Detector to slides, and incubate for 10 min at RT.
2.3.11. Wash slides three times with TBST (5 min each on a shaker).
2.3.12. Add freshly prepared DAB substrate to the sections and incubate until stain develops (10-60 s, according to the instruction).
2.3.13. Rinse sections with water.
2.3.14. Counterstain with Hematoxylin (1-2 min, according to instruction).
2.3.13. Rinse sections with water.
2.3.14. Dehydrate samples using two washes with 100% Ethanol (3 min each), followed by two rinses with Xylene (3 min each).
2.3.15. Mount coverslips on slides using permanent mounting medium.