Required Materials (Not Supplied)

Instrument	LEICA BOND-III Fully Automated IHC and ISH Stainer
Reagents	BOND Epitope Retrieval Solution 2
	BOND Wash Solution 10X
	BOND Polymer Refine Detection DAB
	Deionized Water
Consumables	Microscope Slides
	Coverslips
	Permanent mounting medium and ancillary reagents required for mounting coverslips.

Operation Steps

1. **Dewaxing, Hydration and Antigen Retrieval**: After dewaxing and hydration, tissue section slides (FFPE) are immersed into BOND Epitope Retrieval Solution 2 (1x antigen retrieval solution). After incubate at 100℃ for 25 minutes, let tissue section slides cool down naturally to room temperature within the antigen retrieval solution. Wash the slides 5 times with BOND Wash Solution (1x washing buffer), then incubate at room temperature for 3 minutes.
2. **Blocking**: Incubate with inactivated endogenous peroxidase (Peroxide Block) at room temperature for 5 minutes and wash 3 times with BOND Wash Solution (1x washing buffer).
3. **Primary Antibody Incubation:** Add about 150 µL diluted primary antibody reagent (based on recommend titer, i.e. 1:1000) to each slide and incubate at room temperature for 35 minutes. Then wash the slides 3 times with BOND Wash Solution (1x washing buffer).
4. **Secondary Antibody Incubation:**
   a) Add about 150 µL Post Primary (BOND Polymer Refine Detection DAB) to each slide and incubate at room temperature for 8 minutes, then wash the

slides 2 minutes for 3 times with BOND Wash Solution (1x washing buffer).

b) Add about 150 μL Polymer (BOND Polymer Refine Detection DAB) to each slide and incubate at room temperature for 8 minutes, then wash the slides 2 minutes for 2 times with BOND Wash Solution (1x washing buffer), 2 minutes for once with deionized water.

5. **DAB Color Development**: Add about 150 μL Mixed DAB Refine (BOND Polymer Refine Detection DAB) to each slide and incubate at room temperature for 10 minutes, then wash the slides 3 times with deionized water.
6. **Counterstaining:** Stain each slide with about 150 μL hematoxylin at room temperature for 5 minutes, then wash the slides once with deionized water, once with BOND Wash Solution (1x washing buffer), then once with deionized water.
7. **Mounting**: After dehydration and transparency, use an appropriate amount of mounting medium to mount, observe the slides under a microscope and interpret the results.