

sAC—Immunohistochemistry on Human Tissue using Leica-Bondmax system.

1. **Dewax**—Formalin-fixed paraffin embedded (FFPE) samples are sectioned at 5 μm and baked in a slide oven at 68° C for 2 hours. Sections are dewaxed on the BonMax instrument using the Leica Microsystems' Dewax solution (part number AR 922) for 10 minutes @ 72° C and Dewax solution wash at ambient temperature.
2. The Dewax is followed by 3 100% Alcohol washes (Ethyl Alcohol, ThermoFisher, cat #: 6255) and 3 washes with Wash Buffer (Leica Microsystems, cat #: AR9590).
3. **Retrieval**: Sections are pre-treated with 2 washes with Bond Epitope Retrieval Solution 1, ER1 (Leica Microsystems, cat #: AR9961) followed by 30 minutes epitope retrieval @ 100° C, using the ER1. The pretreated sections are cooled for 12 minutes to ambient temperature.
4. **Staining—Preparation**: Prior to staining, sections are washed 3X with Leica Wash Buffer @ 35° C and then incubate in the Wash Buffer at ambient temperature for 3 minutes.
5. **Staining—Primary Antibody**: Apply and incubate the primary antibody; diluted with Dako Antibody Diluent with Background Reducing Components—cat#: S3022) for 15 minutes.
6. **Staining—Post Primary**: Wash slides 3X in Bond Wash at ambient temperature and incubate sections in Post-Primary AP for 20 minutes (Leica Microsystems Polymer Refine Red Detection Kit, cat#: DS9390).
7. **Staining—Polymer**: Wash slides 3X @ 2 minutes each and apply the Polymer AP for 30 minutes (Leica Microsystems Polymer Refine Red Detection Kit, cat#: DS9390).
8. **Staining (Chromogen)—Mixed Red Refine**: Thoroughly wash off the Polymer AP with Bond Wash Buffer, 3X @ 2 minutes each; then incubate sections in the Wash buffer for 5 minutes and then a final wash. Apply and incubate the Mixed Red Refine for 10 minutes. Re-apply the mixed Red Refine and incubate for 5 more minutes.
9. **Rinsing**—Wash slides 3X in Deionized water
10. **Counterstain**—Apply Hematoxylin and incubate slides for 5-10 minutes.
11. Wash slides with Deionized water, dehydrate in graded alcohol, clear with xylene, and coverslip.