

## Supplementary Table 1. IP1 protocol summary for Ventana Discovery.

- 
- 1 First Sequence [Selected]
  - 2 Baking [Selected]
  - 3 Warmup Slide to [60 Deg C], and Incubate for [8 Minutes] ( Baking )
  - 4 Deparaffinization [Selected]
  - 5 Warmup Slide to [69 Deg C], and Incubate for [8 Minutes] ( Cycle 1 )
  - 6 Incubate for [8 Minutes] ( Cycle 2 )
  - 7 Incubate for [8 Minutes] ( Cycle 3 )
  - 8 Pretreatment [Selected]
  - 9 Cell Conditioning [Selected]
  - 10 CC1 Reservoir [Selected]
  - 11 Warmup Slide to [97 Deg C], and Incubate for 4 Minutes ( Cell Conditioner #1 )
  - 12 CC1 8 Min [Selected]
  - 13 CC1 16 Min [Selected]
  - 14 CC1 24 Min [Selected]
  - 15 CC1 32 Min [Selected]
  - 16 Inhibitor [Selected]
  - 17 DISCOVERY Inhibitor [Selected]
  - 18 Apply One Drop of DISC Inhibitor, and Incubate for [12 Minutes]
  - 19 Antibody [Selected]
  - 20 Apply One Drop of [PREP KIT 10] ( Antibody ), and Incubate for [32 Minutes]
  - 21 Linking Antibody [Selected]
  - 22 Multimer HRP [Selected]
  - 23 Apply One Drop of [OMap anti-Rb HRP] ( Multimer HRP ), and Incubate for [24 Minutes]
  - 24 Open Detection Kit [Selected]
  - 25 Automated Open Detection [Selected]
  - 26 Apply One Drop of [DETECTION 15] ( Detection #23 ), and Incubate for [0 Hr 16 Min]
  - 27 Dual Sequence [Selected]
  - 28 Antibody Denaturation [Selected]
  - 29 Antibody Denature CC2-1 [Selected]
  - 30 Warmup Slide to [100 Deg C], and Incubate for 8 Minutes ( DS CC2 denature )
  - 31 DS Antibody [Selected]
  - 32 Warmup Slide to [36 Deg C] from Very Low Temperatures ( DS Primary Antibody )
  - 33 Apply One Drop of [PREP KIT 14] ( DS Antibody ), and Incubate for [32 Minutes]
  - 34 DS Multimer HRP [Selected]
  - 35 Apply One Drop of [OMap anti-Ms HRP] ( DS Multimer HRP ), and Incubate for [24 Minutes]
  - 36 DS Open Detection Kit [Selected]
  - 37 DS Automated Open Detection [Selected]
  - 38 Apply One Drop of [DETECTION 16] ( Detection #24 ), and Incubate for [0 Hr 16 Min]
  - 39 Triple Stain [Selected]
  - 40 TS Antibody Denaturation [Selected]
  - 41 Antibody Denature CC2-2 [Selected]
  - 42 Warmup Slide to [100 Deg C], and Incubate for 8 Minutes ( TS CC2 denature )
  - 43 TS Antibody [Selected]
  - 44 Warmup Slide to [36 Deg C] from Very Low Temperatures ( TS Primary Antibody )
  - 45 Apply One Drop of [KI-67 (30-9)] ( TS Antibody ), and Incubate for [32 Minutes]
  - 46 TS Multimer HRP [Selected]

## Supplementary Table 1 (continued). IP1 protocol summary for Ventana Discovery.

- 47 Apply One Drop of [OMap anti-Rb HRP] ( TS Multimer HRP ), and Incubate for [24 Minutes]
- 48 TS Open Detection Kit [Selected]
- 49 TS Automated Open Detection [Selected]
- 50 Apply One Drop of [DETECTION 14] ( Detection #25 ), and Incubate for [0 Hr 16 Min]
- 51 Quadruple Stain [Selected]
- 52 QuS Antibody Denaturation [Selected]
- 53 Antibody Denature CC2-3 [Selected]
- 54 Warmup Slide to [100 Deg C], and Incubate for 8 Minutes ( QuS CC2 denature )
- 55 QuS Antibody [Selected]
- 56 Warmup Slide to [36 Deg C] from Very Low Temperatures ( QuS Primary Antibody )
- 57 Apply One Drop of [ANTI-CD20] ( QuS Antibody ), and Incubate for [32 Minutes]
- 58 QuS Multimer HRP [Selected]
- 59 Apply One Drop of [OMap anti-Ms HRP] ( QuS Multimer HRP ), and Incubate for [24 Minutes]
- 60 QuS Open Detection Kit [Selected]
- 61 QuS Automated Open Detection [Selected]
- 62 Apply One Drop of [DETECTION 12] ( Detection #26 ), and Incubate for [0 Hr 16 Min]
- 63 Quintuple Stain [Selected]
- 64 QnS Antibody Denaturation [Selected]
- 65 Antibody Denature CC2-4 [Selected]
- 66 Warmup Slide to [100 Deg C], and Incubate for 8 Minutes ( QnS CC2 denature )
- 67 QnS Antibody [Selected]
- 68 Warmup Slide to [36 Deg C] from Very Low Temperatures ( QnS Primary Antibody )
- 69 Apply One Drop of [anti-CD3 (2GV6)] ( QnS Antibody ), and Incubate for [32 Minutes]
- 70 QnS Multimer HRP [Selected]
- 71 Apply One Drop of [OMap anti-Rb HRP] ( QnS Multimer HRP ), and Incubate for [24 Minutes]
- 72 QnS Open Detection Kit [Selected]
- 73 QnS Automated Open Detection [Selected]
- 74 Apply One Drop of [DETECTION 1] ( Detection #27 ), and Incubate for [0 Hr 16 Min]
- 75 Sextuple Stain [Selected]
- 76 SxS Antibody Denaturation [Selected]
- 77 Antibody Denature CC2-5 [Selected]
- 78 Warmup Slide to [100 Deg C], and Incubate for 8 Minutes ( SxS CC2 Denature )
- 79 SxS Antibody [Selected]
- 80 Warmup Slide to [36 Deg C] from Very Low Temperatures ( SxS Primary Antibody )
- 81 Apply One Drop of [PREP KIT 19] ( SxS Antibody ), and Incubate for [32 Minutes]
- 82 SxS Multimer HRP [Selected]
- 83 Apply One Drop of [OMap anti-Rb HRP] ( SxS Multimer HRP ), and Incubate for [24 Minutes]
- 84 SxS Open Detection Kit [Selected]
- 85 SxS Automated Open Detection [Selected]
- 86 Apply One Drop of [DETECTION 11] ( Detection #28 ), and Incubate for [0 Hr 16 Min]
- 87 Counterstain [Selected]
- 88 Use DW for Counterstain [Selected]
- 89 Apply One Drop of [COUNTERSTAIN 1] ( Counterstain ), and Incubate for [4 Minutes]

## Supplementary Table 2. IP2 protocol summary for Ventana Discovery.

- 1 First Sequence [Selected]
- 2 Baking [Selected]
- 3 Warmup Slide to [60 Deg C], and Incubate for [8 Minutes] ( Baking )
- 4 Deparaffinization [Selected]
- 5 Warmup Slide to [69 Deg C], and Incubate for [8 Minutes] ( Cycle 1 )
- 6 Incubate for [8 Minutes] ( Cycle 2 )
- 7 Incubate for [8 Minutes] ( Cycle 3 )
- 8 Pretreatment [Selected]
- 9 Cell Conditioning [Selected]
- 10 CC1 Reservoir [Selected]
- 11 Warmup Slide to [97 Deg C], and Incubate for 4 Minutes ( Cell Conditioner #1 )
- 12 CC1 8 Min [Selected]
- 13 CC1 16 Min [Selected]
- 14 CC1 24 Min [Selected]
- 15 CC1 32 Min [Selected]
- 16 CC1 40 Min [Selected]
- 17 CC1 48 Min [Selected]
- 18 CC1 56 Min [Selected]
- 19 CC1 64 Min [Selected]
- 20 CC1 72 Min [Selected]
- 21 CC1 80 Min [Selected]
- 22 CC1 88 Min [Selected]
- 23 CC1 92 Min [Selected]
- 24 Inhibitor [Selected]
- 25 DISCOVERY Inhibitor [Selected]
- 26 Apply One Drop of DISC Inhibitor, and Incubate for [12 Minutes]
- 27 Antibody [Selected]
- 28 Apply One Drop of [PREP KIT 12] ( Antibody ), and Incubate for [60 Minutes]
- 29 Multimer HRP [Selected]
- 30 Apply One Drop of [OMap anti-Rb HRP] ( Multimer HRP ), and Incubate for [24 Minutes]
- 31 Open Detection Kit [Selected]
- 32 Automated Open Detection [Selected]
- 33 Apply One Drop of [DETECTION 15] ( Detection #23 ), and Incubate for [0 Hr 16 Min]
- 34 Dual Sequence [Selected]
- 35 Antibody Denaturation [Selected]
- 36 Antibody Denature CC2-1 [Selected]
- 37 Warmup Slide to [100 Deg C], and Incubate for 8 Minutes ( DS CC2 denature )
- 38 DS Antibody [Selected]
- 39 Warmup Slide to [36 Deg C] from Very Low Temperatures ( DS Primary Antibody )
- 40 Apply One Drop of [PREP KIT 16] ( DS Antibody ), and Incubate for [32 Minutes]
- 41 DS Multimer HRP [Selected]
- 42 Apply One Drop of [OMap anti-Rb HRP] ( DS Multimer HRP ), and Incubate for [24 Minutes]
- 43 DS Open Detection Kit [Selected]
- 44 DS Automated Open Detection [Selected]
- 45 Apply One Drop of [DETECTION 16] ( Detection #24 ), and Incubate for [0 Hr 16 Min]
- 46 Triple Stain [Selected]

## Supplementary Table 2 (continued). IP2 protocol summary for Ventana Discovery.

- 47 TS Antibody Denaturation [Selected]
- 48 Antibody Denature CC2-2 [Selected]
- 49 Warmup Slide to [100 Deg C], and Incubate for 8 Minutes ( TS CC2 denature )
- 50 TS Antibody [Selected]
- 51 Warmup Slide to [36 Deg C] from Very Low Temperatures ( TS Primary Antibody )
- 52 Apply One Drop of [PREP KIT 9] ( TS Antibody ), and Incubate for [32 Minutes]
- 53 TS Multimer HRP [Selected]
- 54 Apply One Drop of [OMap anti-Ms HRP] ( TS Multimer HRP ), and Incubate for [24 Minutes]
- 55 TS Open Detection Kit [Selected]
- 56 TS Automated Open Detection [Selected]
- 57 Apply One Drop of [DETECTION 14] ( Detection #25 ), and Incubate for [0 Hr 16 Min]
- 58 Quadruple Stain [Selected]
- 59 QuS Antibody Denaturation [Selected]
- 60 Antibody Denature CC2-3 [Selected]
- 61 Warmup Slide to [100 Deg C], and Incubate for 8 Minutes ( QuS CC2 denature )
- 62 QuS Antibody [Selected]
- 63 Warmup Slide to [36 Deg C] from Very Low Temperatures ( QuS Primary Antibody )
- 64 Apply One Drop of [PREP KIT 14] ( QuS Antibody ), and Incubate for [32 Minutes]
- 65 QuS Multimer HRP [Selected]
- 66 Apply One Drop of [OMap anti-Ms HRP] ( QuS Multimer HRP ), and Incubate for [24 Minutes]
- 67 QuS Open Detection Kit [Selected]
- 68 QuS Automated Open Detection [Selected]
- 69 Apply One Drop of [DETECTION 11] ( Detection #26 ), and Incubate for [0 Hr 16 Min]
- 70 Quintuple Stain [Selected]
- 71 QnS Antibody Denaturation [Selected]
- 72 Antibody Denature CC2-4 [Selected]
- 73 Warmup Slide to [100 Deg C], and Incubate for 8 Minutes ( QnS CC2 denature )
- 74 QnS Antibody [Selected]
- 75 Warmup Slide to [36 Deg C] from Very Low Temperatures ( QnS Primary Antibody )
- 76 Apply One Drop of [PREP KIT 18] ( QnS Antibody ), and Incubate for [32 Minutes]
- 77 QnS Multimer HRP [Selected]
- 78 Apply One Drop of [OMap anti-Ms HRP] ( QnS Multimer HRP ), and Incubate for [24 Minutes]
- 79 QnS Open Detection Kit [Selected]
- 80 QnS Automated Open Detection [Selected]
- 81 Apply One Drop of [DETECTION 12] ( Detection #27 ), and Incubate for [0 Hr 16 Min]
- 82 Sextuple Stain [Selected]
- 83 SxS Antibody Denaturation [Selected]
- 84 Antibody Denature CC2-5 [Selected]
- 85 Warmup Slide to [100 Deg C], and Incubate for 8 Minutes ( SxS CC2 Denature )
- 86 SxS Antibody [Selected]
- 87 Warmup Slide to [36 Deg C] from Very Low Temperatures ( SxS Primary Antibody )
- 88 Apply One Drop of [anti-CD3 (2GV6)] ( SxS Antibody ), and Incubate for [32 Minutes]
- 89 SxS Multimer HRP [Selected]
- 90 Apply One Drop of [OMap anti-Rb HRP] ( SxS Multimer HRP ), and Incubate for [24 Minutes]
- 91 SxS Open Detection Kit [Selected]
- 92 SxS Automated Open Detection [Selected]
- 93 Apply One Drop of [DETECTION 1] ( Detection #28 ), and Incubate for [0 Hr 16 Min]
- 94 Counterstain [Selected]
- 95 Use DW for Counterstain [Selected]
- 96 Apply One Drop of [COUNTERSTAIN 1] ( Counterstain ), and Incubate for [4 Minutes]