

## IHC VENTANA DISCOVERY XT PROTOCOL

The recommended protocol for immunohistochemistry staining using Ventana Discovery XT is described below. Ventana Discovery XT is a closed system and all steps are performed in the instrument (from deparaffinization step to counterstaining).

### DEPARAFFINIZATION

Paraffin sections of 4 µm thickness are baked overnight at 50°C. The deparaffinization step is done in the Ventana Discovery XT platform using EZ prep solution.

### STANDARD ANTIGEN RETRIEVAL METHOD

The standard antigen retrieval method is Heat Induced Epitope Retrieval (HIER) in Tris-EDTA buffer pH 7.8 at 95°C for 44 min (standard CC1) and is performed in the Ventana Discovery XT.

If expected results are not achieved test a different time or use Citrate-based buffer pH 6.0 or Protease antigen retrieval instead.

### BLOCKING STEPS

For blocking endogenous peroxides and protein, these steps are done in the Ventana Discovery XT platform using Inhibitor CM.

**NOTE:** The specified working dilutions of the primary antibodies are to be considered as a guideline only. Optimal dilutions must be determined by end user.

### IMMUNOHISTOCHEMICAL STAINING PROTOCOL, VENTANA DISCOVERY XT

Washing buffer between the steps is Reaction buffer. Apply 100 µl volume of primary and secondary antibodies.

1. Tissue Sample, Paraffin
2. Deparaffinization in EZ prep 75°C 8 minutes.
3. Cell Conditioning using Conditioner #1, Standard CC1, 95°C 44 minutes
4. Block with Inhibitor CM, 37°C 4 minutes
5. No Heat
6. Incubation with primary antibody for 60 minutes
7. Apply one drop of OmniMap anti-Rb HRP and incubate for 16 minutes\*
8. Apply one drop of OmniMap anti-Ms HRP and incubate for 16 minutes\*\*
9. Apply One drop of DAB CM and One Drop H<sub>2</sub>O<sub>2</sub> CM, incubate 8 minutes
10. Apply One drop of Copper CM, incubate 5 minutes
11. Counterstain with Hematoxylin, incubate for 8 minutes
12. Post counterstain with Bluing Reagent, incubate for 8 minutes
13. Slide Cleaning

Wash the slides in warm tap water with detergent and dehydrate in graded ethanol and xylene. Coverslip the slides in permanent mounting media.

\* For Triple A Polyclonals™ use OmniMap anti-Rt HRP.

\*\* For PrecisA Monoclonals™ use OmniMap anti-Ms HRP.

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### REAGENTS

For immunohistochemistry, the following reagents are commercially available from Roche, Ventana Medical Systems, Tucson, AZ, USA.

- EZ Prep Solution, 950-100
- Reaction buffer, Tris-based buffer pH 7.6-7.8, 950-300
- Liquid Coverslip, LCS, 650-010
- Cell Conditioning Solution, CC1, Tris-EDTA based buffer pH 7.8, 950-124
- RiboCC Solution, CC2, Citrate based buffer pH 6.5, 760-107
- Protease 1, 760-2018
- Antibody Dilution Buffer, ADB250
- ChromoMap DAB kit, 760-159
- OmniMap anti-Ms HRP, 760-4310
- OmniMap anti-Rt HRP, 760-4311
- Hematoxylin, 760-2021
- Bluing Reagent, 760-2037

In addition,

- EtOH from Histolab, Gothenburg. Sweden
- Pertex, 00811, Histolab, Gothenburg. Sweden
- Rabbit anti-Mouse IgG1 + IgG2 + IgG3 (3024-1) [M204-3] ab133468, Abcam

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