

## **IHC Protocol:**

**-BioGenex i6000 with off-line HIER**

**-Leica BOND RX with on-line HIER**

BioGenex with off-line HIER:

### IHC Reagents

- Xylene (EM Science, Cat # UN1307)
- Ethanol (AAPER alcohol and Chemical Co)
- Wash Buffer (DAKO, Cat# S3006)
- Target Retrieval Solution, 10X (Dako, Cat#S1699)
- Novolink Max Polymer Detection System (Leica, Cat# RE7260-CE)
- Peroxidase block (Dako Cat# S2003)
- Protein block (Dako Cat# X0909)
- Common antibody Diluent (BioGenex, Cat# HK156-5K)
- DAB (DAKO Cat# K3468)
- 28-8 rabbit monoclonal antibody (Abcam # ab205921, final concentration 2 µg/mL)
- Isotype control rabbit monoclonal antibody (Abcam # ab172730, final concentration 2 µg/mL)

### Equipment:

- Leica autostainer ST5020
- BioGenex i6000 Autostainer
- BioCare Medical Decloaking Chamber™ Plus
- Leica Auto Coverslipper CV5030

BioGenex i6000 with off-line HIER:

### Assay Procedure

#### HIER Method:

- Deparaffinization & rehydration, on Leica autostainer ST5020
- 2X Xylene at 5 min. each; 2X of 100% Ethanol at 2 min. each, 2X of 95% Ethanol at 2 min. each, 2X of 70% Ethanol at 2 min each; dH<sub>2</sub>O, 2 min
- Antigen retrieval by using Biocare Medical Decloaking Chamber Plus
- Dako AR6, heated to 110 °C (P1) for 10 min, then move to the next step (P2)
- FAN ON at 95°C; FAN OFF at 90 °C)
- Cool slide at room temp for 15 min, Rinse with water ~ 1min

BioGenex i6000 with off-line HIER:

Assay Procedure (continued)

IHC Method:

- Apply the “Peroxidase Block” for 10 min. Wash 3X with IHC wash buffer
- Apply the “Protein Block” to the slides, and incubate 20 min. at RT. Wash 3X with IHC wash buffer
- Apply the pre-diluted antibodies to the slides (see Reagents). Incubate 1 hr at R.T.
- Wash 3X with IHC wash buffer
- Add the “Post Primary Block” (NovoLink Kit) to the slides and incubate for 30 min.
- Wash 3X with IHC wash buffer
- Add the “NovoLink Polymer” (NovoLink Kit) to the slides, incubate for 45 min.
- Rinse 3X with IHC wash buffer
- Add the DAB chromogen substrate, Develop 5 mins,
- Wash slides by rinsing with dH2O for 5X at R.T.
- Counterstain with Hematoxylin for 1 min at R.T.
- Wash slides by rinsing with dH2O for 5X at R.T.

BioGenex i6000 with off-line HIER:

Assay Procedure (continued):

- De-hydration and coverslipping:
- Remove the slides from BioGenex i6000 autostainer
- Set up slides on Leica autostainer ST5020
- Dehydrate 2 min each: 1X with 70% Ethanol, 1X with 95% Ethanol, then 3X with 100% Ethanol
- Wash in Xylene for 3X at 2 min. each
- Coverslip with Cytoseal Mounting Medium in Leica CV5030 coverslipper.

## Leica BOND RX with on-line HIER Protocol

### Leica BOND RX with on-line HIER:

- Assay Procedure:
- IHC protocol reagents:
- Bond Dewax Solution (Leica, Cat# AR9222)
- Bond™
- Epitope Retrieval 1 (Leica, Cat# AR9961)
- Bond™
- Wash Solution (Leica, Cat# AR9590)
- Leica BOND Polymer Refine Detection kit (Leica, Cat#DS9800)
- Common Antibody Diluent (BioGenex, Cat# HK156-5K)
- 28-8 rabbit monoclonal antibody (Abcam # ab205921, final concentration 2 µg/mL)
- Isotype control rabbit monoclonal antibody (Abcam # Ab172730, final concentration 2 µg/mL)

### Equipment:

- Leica BOND RX autostainer

### Method:

- Heat and De-wax on Leica BOND RX
- Antigen retrieval on Leica BOND RX at 100°C for 30 min using ER1 (Citra buffer, pH6 from Leica)
- Apply the “Peroxidase block” (Refine Kit) for 10 min., followed by 3X rinse with IHC wash buffer
- Apply the diluted antibodies to the slides (see reagents). Incubate 1 hr. at R.T.
- Wash 3X with IHC wash buffer
- Add the “Post Primary Block” (Refine Kit) to the slides, incubate for 30 min.
- Wash 3X with IHC wash buffer
- Add the “NovoLink Polymer” (Refine Kit) to the slides, incubate for 30 min.
- Wash 3X with IHC wash buffer
- Add the DAB chromogen substrates (Refine Kit), Develop 10 mins,
- Wash slides by rinsing with dH<sub>2</sub>O for 5X at R.T.
- Counterstain with Hematoxylin (Refine Kit) for 8 min. at R.T.
- Wash slides by rinse with dH<sub>2</sub>O for 5X at R.T.

Leica BOND RX with on-line HIER:

De-hydration and coverslipping:

- Remove the slides from Leica BOND RX
- Set up slides on Leica autostainer ST5020
- Dehydrate 2 min. each, 1 X with 70% Ethanol, 1X with 95% Ethanol, then 2X with
- 100% Ethanol
- Wash 2X in Xylene for 2 min. each
- Coverslip with Cytoseal Mounting Medium in Leica CV5030 coverslipper