

Supplemental Figure S2. ARv7, AR, SMA Multiplex Staining Protocol

Automated Immunohistochemistry performed on the Ventana DiscoveryXT BioMarker Platform & Partial Manual Stain

Deparaffinization was carried out on the instrument, as was heat-induced epitope retrieval in the form of “cell conditioning” with CC2 buffer (Ventana #760-107), a citrate based buffer, for approximately 60 minutes at 100°C.

Primary antibody Arv7 diluted 1:100 in DaVinci Green (BioCare Medical #PD900H) incubated for 1 hr with no heat.

Rinse with Reaction Buffer (Ventana #950-300)

Discovery UltraMap anti-Mouse HRP (Ventana #760-4313) incubated for 12 min at 37 degrees.

Rinse with Reaction Buffer (Ventana #950-300)

Discovery ChromoMap DAB detection (Ventana #760-159) was applied for preset time

Rinse with Reaction Buffer (Ventana #950-300)

Denature with Biocare kit.

Rinse with dH₂O

Rinse with Reaction Buffer (Ventana #950-300)

Primary antibody AR diluted 1:100 in DaVinci Green (BioCare Medical #PD900H) incubated for 2 hrs with no heat.

Rinse with Reaction Buffer (Ventana #950-300)

Discovery anti-Rabbit HQ (Ventana #760-4815) incubated for 12 min at 37 degrees.

Rinse with Reaction Buffer (Ventana #950-300)

Discovery Purple detection kit (Ventana #760-229) was applied for preset time

Rinse with dH₂O

Transfer to TBS-T

Primary antibody SMA diluted 1:400 in DaVinci Green (BioCare Medical #PD900H) incubated for 30 minutes at ambient temperature.

Rinse with TBS-T

Mach 2 Mouse HRP-Polymer (Biocare Medical MHRP520G) incubated 30 minutes at ambient temperature.

Rinse with TBS-T

Vina Green Chromogen (Biocare Medical BRR807AH) incubated for 5 minutes at room temperature.

Rinse with TBS-T.

Rinse with dH₂O

Harris hematoxylin counterstain 1:5 for 30 sec

Rinse with dH₂O

Dehydrated

Xylene

Coverslip