



The figure (40x) shows the negative control on DAXX staining. The tissue used was adult liver. There is no reactivity on the hepatocytes



The figure (40x) shows the positive control on DAXX staining. The tissue used was cerebral cortex. It shows strong nuclear and cytoplasmic staining in the tumor cells

Protocol for immunohistochemistry:

The entire procedure was performed at BenchMark ULTRA IHC/ISH Staining Module (Ventana Medical Systems, Tucson). The chosen procedure is U OptiView DAB IHC v5 (v1.00.0117).

- (1) The tissue block was chosen and deparaffinized. Slides were warmed up to 72 degree Celsius.
- (2) Cell conditioning
- (3) CC (cell condition) 1 8 min
- (4) CC1 16 min
- (5) CC1 24 min
- (6) CC1 32 min
- (7) Pre Primary Peroxidase inhibit
- (8) Primary antibody (Anti-DAXX (Sigma Life Science; HPA008736, 1:100) incubated at room temperature (25 degree Celsius for 30 mins)
- (9) Apply Coverslip, One drop of [PREP KIT 84] (Antibody), and incubate for 40 min
- (10) OptiView Amplification
- (11) Apply one drop of OV AMP (Optiview amplified-kit) H2O2 and One drop of OV AMPLIFIER, APPLY coverslip, incubate for 4 minutes
- (12) Apply one drop of OV AMP MULTIMER (Optiview amplified HRP multimer), Apply coverslip, and incubate for 4 minutes
- (13) Counterstain
- (14) Apply one drop of hematoxylin (counterstain), apply coverslip, and incubate for 4 minutes
- (15) Post counterstain
- (16) Apply one drop of bluing reagent (post counterstain), apply coverslip and incubate for 4 minutes

Abbreviations:

Cell condition 1 (**Catalog Number:** 950-124)

Ventana Medical Systems, Inc. (Ventana) Cell Conditioning Solution (CC1) is a pre diluted solution used as a pretreatment step in the processing of tissue samples for immunohistochemistry (IHC) reactions carried out on VENTANA BenchMark and BenchMark XT automated slide stainers.

PREP KIT 84 (**Catalog Number:** 783-3040)

Inline User-Fillable Kit and Prep Kit are used to deliver reagents and solutions on the VENTANA BenchMark Series automated slide stainers (BenchMark, BenchMark GX, BenchMark XT, and BenchMark ULTRA). The dispenser in the kit is intended for single use only. Ventana has not validated the cleaning of these dispensers and re-use with a second reagent.

OV AMP (Optiview amplified-kit)(**Catalog Number:** 860-099)

OptiView Amplification Kit may be used in conjunction with the OptiView DAB IHC Detection Kit (OptiView Detection Kit) to increase the staining intensity of mouse and rabbit primary antibodies. The kit is to be used for qualitative staining of formalin-fixed, paraffin-embedded tissue, frozen tissue, or cytological preparations on VENTANA BenchMark Advanced Staining automated slide stainers with VENTANA primary antibodies and ancillary reagents, visualized by light microscopy.