Validation Report #029457

Validation Date: 01/16/14

Summary

Antigen	Aquaporin 2 (AQP2)
Catalog number	ABIN707576
Supplier	Bioss
Supplier catalog number	bs-4611r
Lot number	999994W
Method validated	Immunohistochemistry
Laboratory	Immunohistochemistry Core, NYU Langone
Validation number	29457
Positive Control	• Human kidney tubules • Human breast ductal epithelium
Negative Control	• Human kidney glomeruli and stromal tissue • Human breast mesenchymal tissue
Notes	Strong signal was observed in positive control tissues and not in negative control tissues.

Full Methods

Primary Antibody
- Antibody: Aquaporin 2
- Catalog number: ABIN707576
- Supplier: Bioss
- Supplier number: Bs-4611R
- Batch number: 999994W

Isotype Control Antibody
- Antibody: Rabbit IgG isotype control
- Catalog number: 790-4795
- Supplier: Ventana Medical Systems
- Lot number: C11487

Secondary Antibody
- Antibody: Biotinylated goat anti-rabbit/anti-mouse (Kit)
- Catalog number: 760-091
- Supplier: Ventana Medical Systems
- Lot number: D05923BA

Additional Information

Detection kit information
- Type: iVIEW Streptavidin Peroxidase DAB
- Catalog number: 760-091
- Supplier: Ventana Medical Systems
- Batch number: D05923A

Controls

Tissues stained came from a human FFPE tissue microarray (12-003d).
- Positive control: Ductal epithelium from human kidney and breast tissue (specimen known to contain the target protein).
- Negative Control: Stroma/mesenchyme from human kidney and breast tissue (specimen known to not contain the target protein).
- Primary antibody isotype control: Ductal epithelium from human kidney and breast tissue (specimen known to contain the target protein) treated with primary antibody isotype control instead of the primary antibody.
- Secondary antibody only control: Ductal epithelium from human kidney and breast tissue (specimen known to contain the target protein) treated with secondary antibody only (no primary antibody).

Protocol

Immunohistochemistry was performed on a Ventana NexES automated platform, instrument manufacturer specific reagents are italicized.

1. Slides were preheated in convection oven at 60°C for 30 min
2. Deparaffinization procedure:
    - 3 changes of Xylene, 5 min each
    - 3 changes of 100% Ethanol, 3 min each
    - 3 changes of 95% Ethanol, 3 min each
    - Rinsed in distilled water, 3 changes
3. Heat retrieval procedure
    - Slides retrieved in 10.0 mM Citrate, pH6.0 in a 1000W microwave oven (~100°C) for 15 min.
    - Slides were allowed to cool (in citrate) for 30 min.
    - Slides were washed x 3 in Distilled water
4. NexES instrument procedure, iVIEW DAB paraffin protocol (*abridged*):
    - Slide chamber warmed to 37°C
5. Slides rinsed with reaction buffer x3
6. *iVIEW Inhibitor (H2O2)* applied and incubated for 4 min

7. Slides rinsed with *reaction buffer*
8. Antibody Application
    - Primary antibody diluted 1:250 in PBS (100 µL applied/slide)
    - Ventana Isotype control applied neat
    - Slides Incubated overnight at room temperature (~12 hours ~25°C)

9. Slides rinsed with *reaction buffer* x3
10. *iVIEW Biotinylated IgG* applied and incubated for 8 min
11. Slides rinsed with *reaction buffer*
12. *iVIEW Streptavidin-Horseradish Peroxidase* applied and incubated for 8 min
13. Slides rinsed with *reaction buffer*
14. *iVIEW DAB/H2O2* applied and incubated for 8 min
15. Slides rinsed with *reaction buffer*
16. *iVIEW Copper* applied and incubated for 4 min
17. Slides rinsed with *reaction buffer*
18. Slides washed in Dawn Detergent/tap water
19. Counterstain Procedure
    - Hematoxylin (Leica 560 MX) 30 seconds
    - Slides washed in tap water, 1 min
    - Decolorized (10% Acetic Acid in 70% ethanol), 1 min
    - Slides washed in tap water, 1 min
    - Bluing (Austin Clear Ammonia), 1 min
    - Slides washed in tap water, 1 min

20. Dehydration/coverslipping procedure:
    - 3 changes of 95% Ethanol, 3 min each
    - 3 changes of 100% Ethanol, 3 min each
    - 3 changes of Xylene, 5 min each
    - Mounted with Permount

21. Imaging: Leica SCN 400F Whole Slide Scanner with Digital Image Hub and Leica Slidepath software

Experimental Notes

Deviations from protocol/procedure supplied by manufacturer.

- Step 1: Heated tissue 60°C for 30 minutes; manufacturer heats for 45 minutes.
- Step 2: No ethanol wash was performed during deparaffinization; manufacturer includes 1 wash of 80% ethanol for 3 minutes.
- Step 3.1: Slides heated for 15 minutes; manufacturer provides a range of 15-20 minutes.
- Step 3.2: Slides cooled for 30 minutes; manufacturer cools for 20 minutes.
- Step 4: Italicized reagents and incubation time are fixed instrument parameters.
- Step 5: Secondary species-specific serum block not used; manufacturer blocks with 5% normal goat serum for 2 hours.

Figures

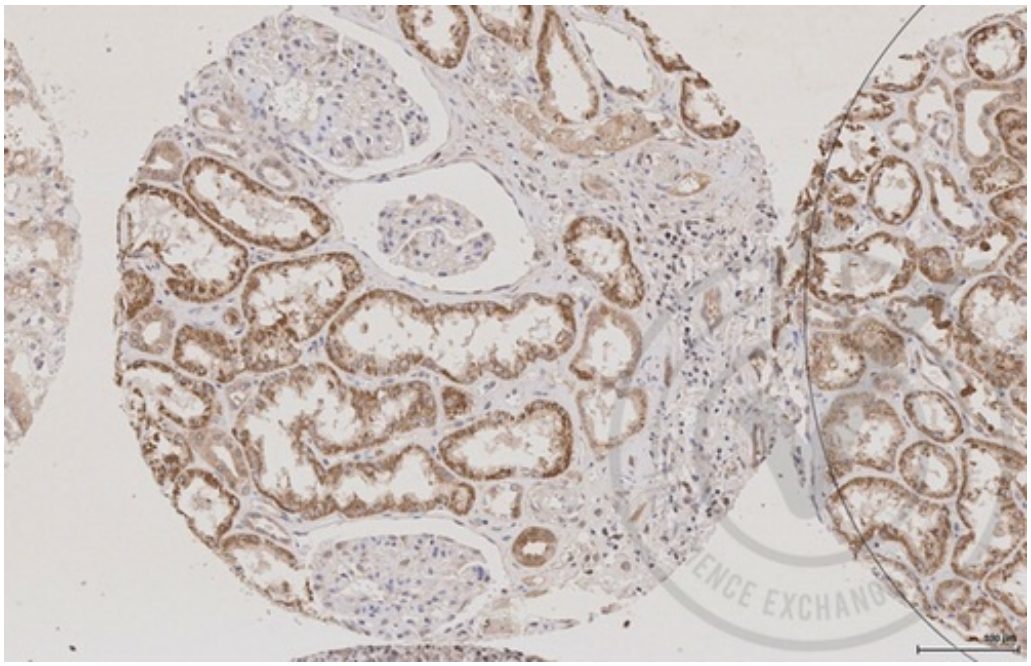

Figure 1: Human kidney stained with AQP2 (brown).

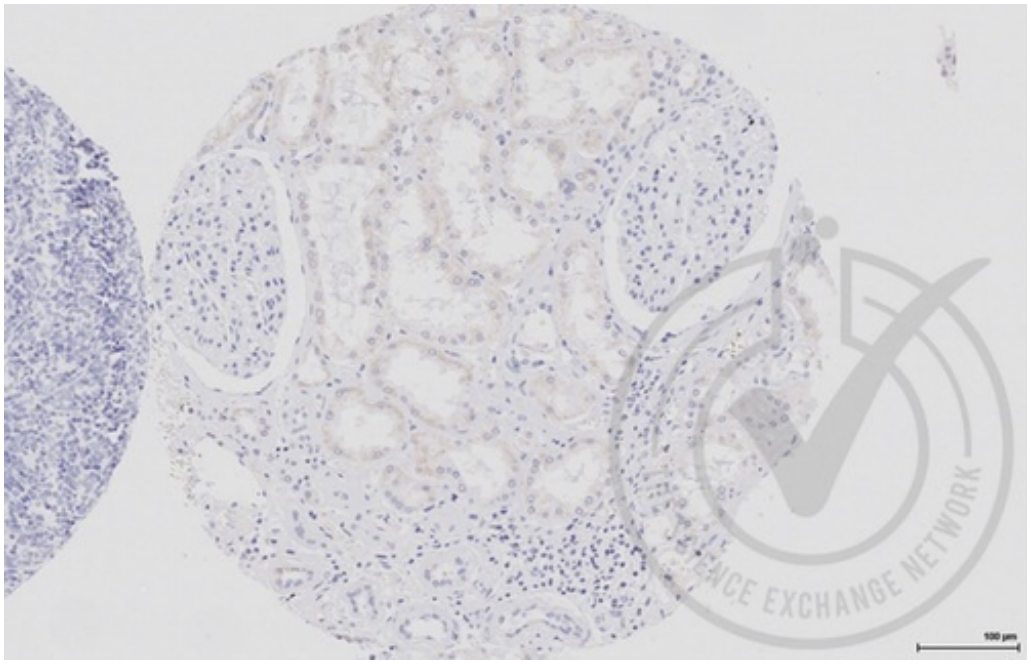

Figure 2. Human kidney stained with isotype control antibody (brown).

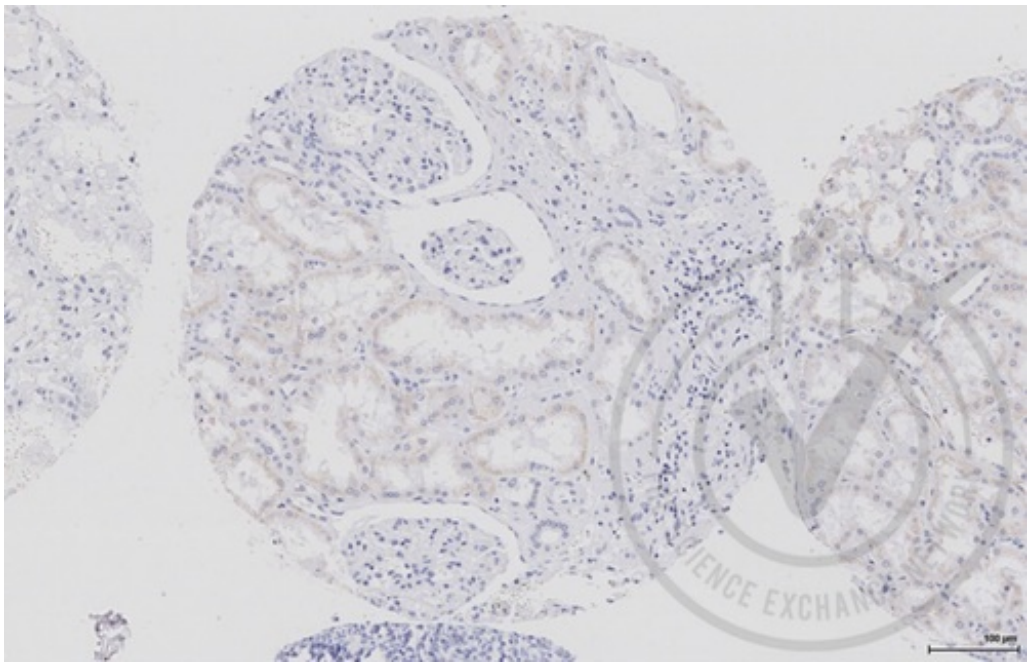

Figure 3: Human kidney stained only with secondary antibody (primary antibody omitted) (brown).

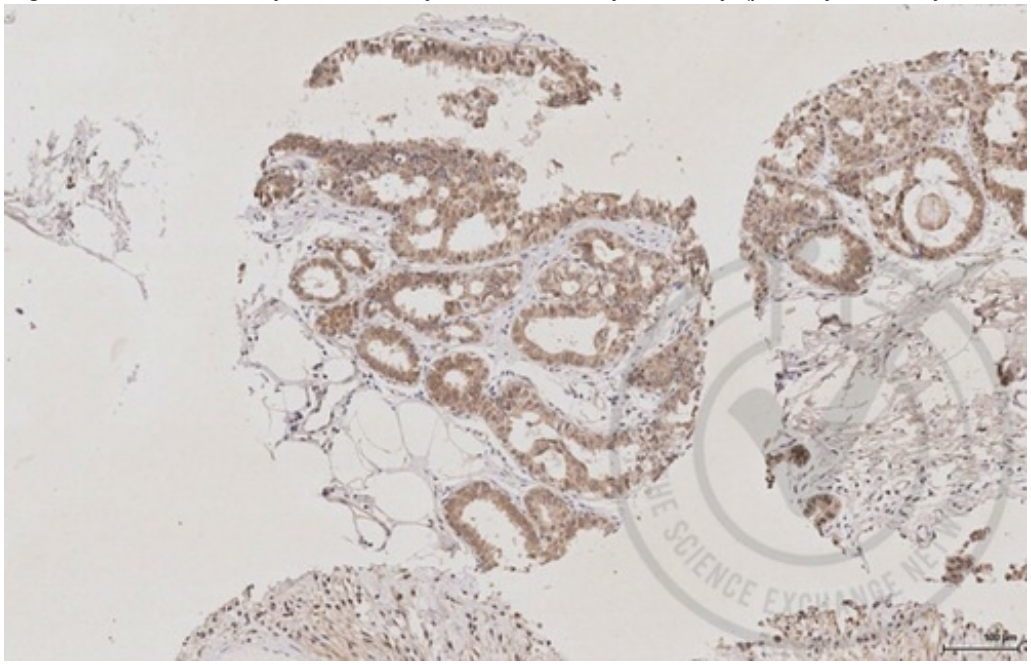

Figure 4: Human breast stained with AQP2 (brown).

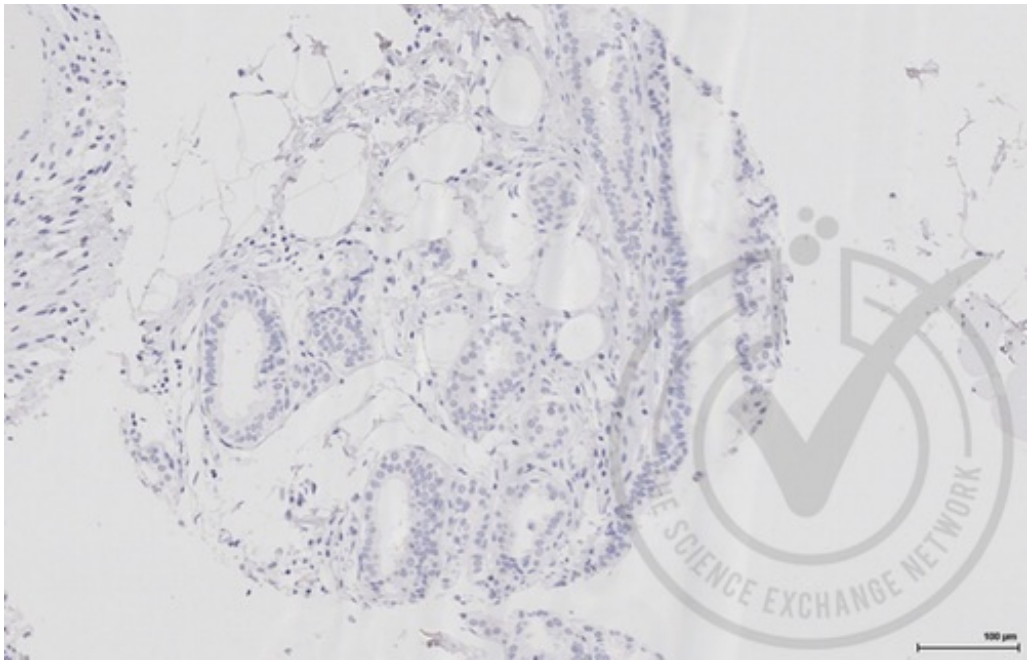

Figure 5. Human breast stained with isotype control antibody (brown).

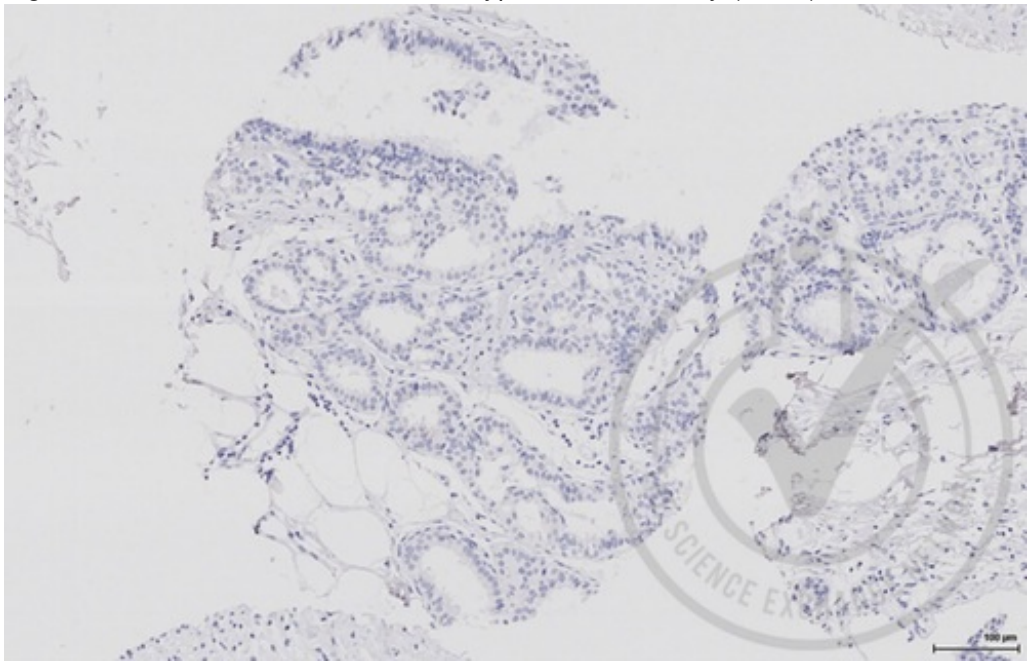

Figure 6: Human breast stained only with secondary antibody (primary antibody omitted) (brown).