Validation Report #029577

Validation Date: 01/19/14

Summary

Antigen	Tight Junction Protein 1
Catalog number	ABIN675024
Supplier	Bioss
Supplier catalog number	bs-1329r
Lot number	130913
Method validated	Immunohistochemistry
Laboratory	Immunohistochemistry Core, NYU Langone
Validation number	29577
Positive Control	Testis
Negative Control	See Controls section for negative controls
Notes	Signal was detected in positive control tissue, and not detected in a tissue expected to express very low levels of the target antigen.

Full Methods

Primary Antibody
- Antibody: human Tight Junction Protein 1 (Zona Occludens 1) (TJP1)
- Catalog number: ABIN675024
- Supplier: Bioss
- Supplier number: bs-1329r
- Lot number: 130913

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
- Lot number: D05923A

Controls

Tissues stained came from a human formalin-fixed paraffin embedded (FFPE) tissue microarray (12-003d):
- Positive control (specimen known to contain the target protein): human testis, which is expected to express high levels of the antigen.
- Negative Control (specimen known to not contain the target protein): Indeterminate; protein located on cytoplasmic membrane surface of intercellular tight junctions. The protein may be involved in signal transduction at cell-cell junctions. Expression is expected to be widespread. Thymus, expected to express much lower levels of the antigen as compared to testis, is shown to demonstrate a tissue with no detectable expression.
- Primary antibody isotype control: Testis (specimen known to contain the target protein) treated with primary antibody isotype control instead of the primary antibody.
- Secondary antibody only control: Testis (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 minutes
2. Deparaffinization procedure:
    - 3 changes of Xylene, 5 minutes each
    - 3 changes of 100% Ethanol, 3 minutes each
    - 3 changes of 95% Ethanol, 3 minutes 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 minutes.
    - Slides were allowed to cool (in citrate) for 30 minutes.
    - 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* x 3
6. *iVIEW Inhibitor (H2O2)* applied and incubated for 4 minutes
7. Slides rinsed with *reaction buffer*
8. Antibody Application
    - Primary antibody diluted 1:250 in PBS (100 microliters 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 minutes
11. Slides rinsed with *reaction buffer*
12. *iVIEW Streptavidin-Horseradish Peroxidase* applied and incubated for 8 minutes
13. Slides rinsed with *reaction buffer*
14. *iVIEW DAB/H2O2* applied and incubated for 8 minutes
15. Slides rinsed with *reaction buffer*
16. *iVIEW Copper* applied and incubated for 4 minutes
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 minute
    - Decolorized (10% Acetic Acid in 70% ethanol), 1 minute
    - Slides washed in tap water, 1 minute
    - Bluing (Austin Clear Ammonia), 1 minute
    - Slides washed in tap water, 1 minute

20. Dehydration/coverslipping procedure:
    - 3 changes of 95% Ethanol, 3 minutes each
    - 3 changes of 100% Ethanol, 3 minutes each
    - 3 changes of Xylene, 5 minutes 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 (attached).

- 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 were heated for 15 minutes; manufacturer provides a range of 15-20 minutes.
- Step 3.2: Slides were 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.
- Step 8.1: Antibody diluted in PBS at 1:250; manufacture did not recommend diluent or dilution.
- Step 8.2.1: Primary antibody incubated at room temperature overnight; manufacturer incubates overnight 4°C with agitation.

Tissue Interpretation (limited):

- TJP1: Under the staining parameters described above, testis stained weakly (ducts) positive (Figure 1). Substantial signal detected in limited number of other tissues, including: breast, normal (NOS); pancreatic cancer (NOS), and stomach, normal (NOS). Most tissues showed low level of specific signal. Thymus did not have any detectable signal (Figure 4).
- I-NC (Isotype negative control): No signal detected
- B-NC (Blank negative control): No signal detected

Signal Localization:

- Signal to noise was adequate with cytoplasmic, nuclear subcellular localization observed. Rare inner-membrane and no distinct membrane signal observed.

Figures

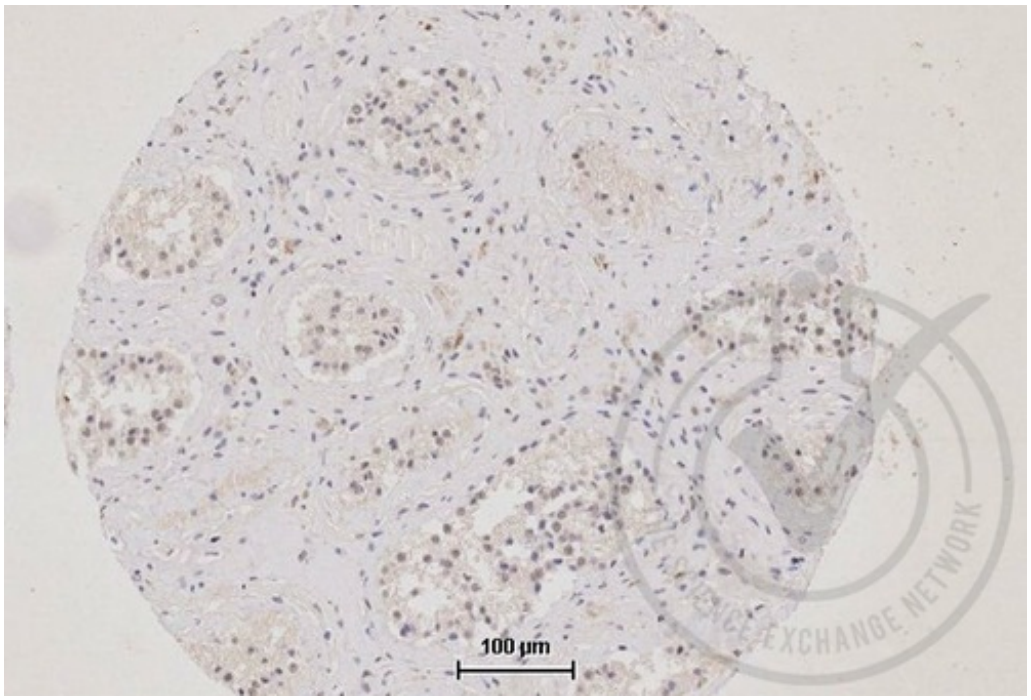

Figure 1: TJP1 immunostaining of human testis (brown). Counterstain in blue.

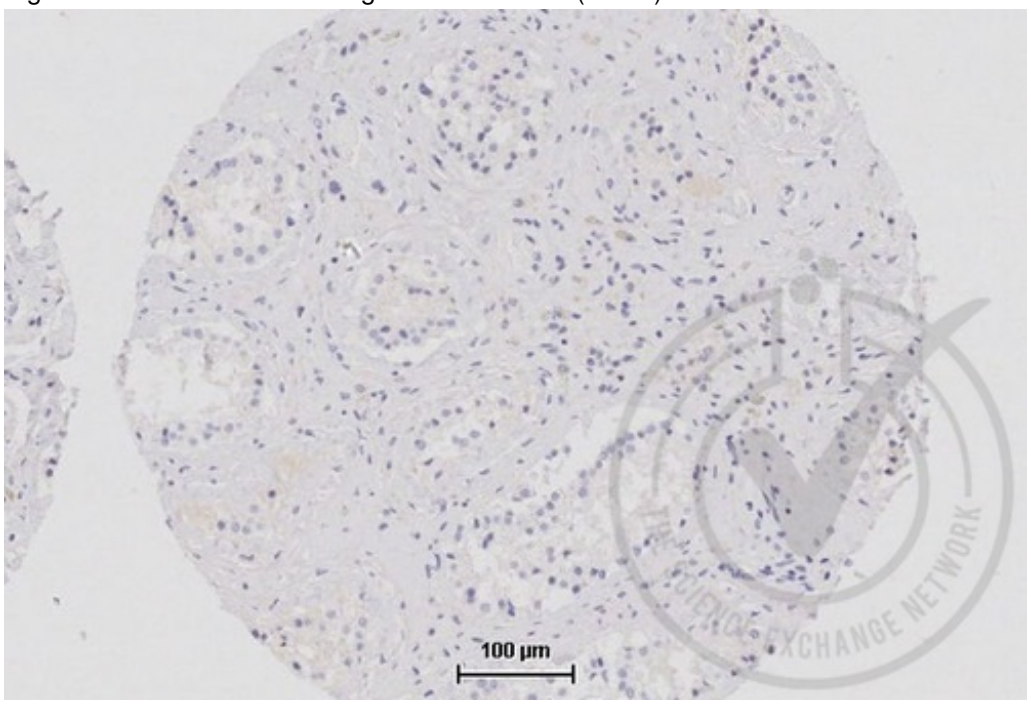

Figure 2: Isotype control immunostaining of human testis (brown). Counterstain in blue.

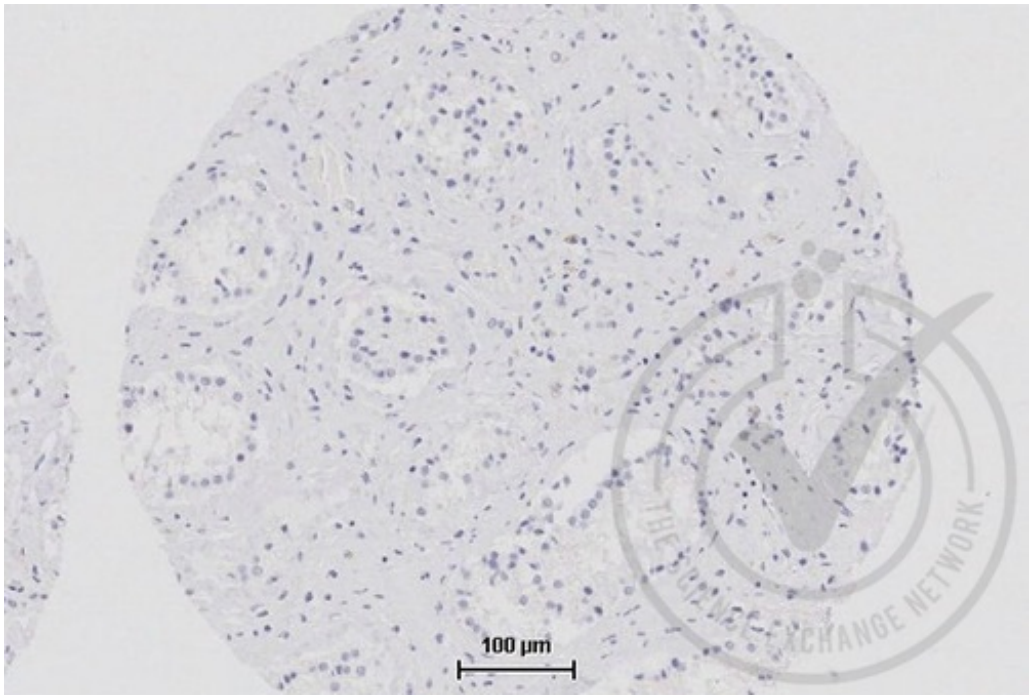

Figure 3: Secondary only control immunostaining of human testis (brown). Counterstain in blue.

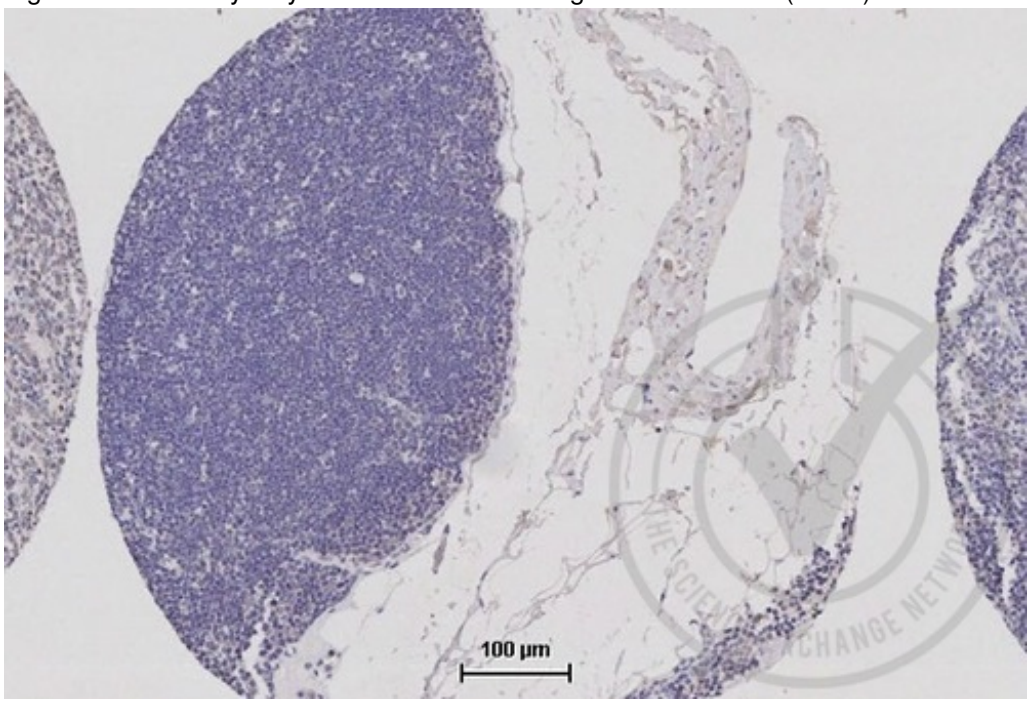

Figure 4: TJP1 immunostaining of human thymus (brown). Counterstain in blue.

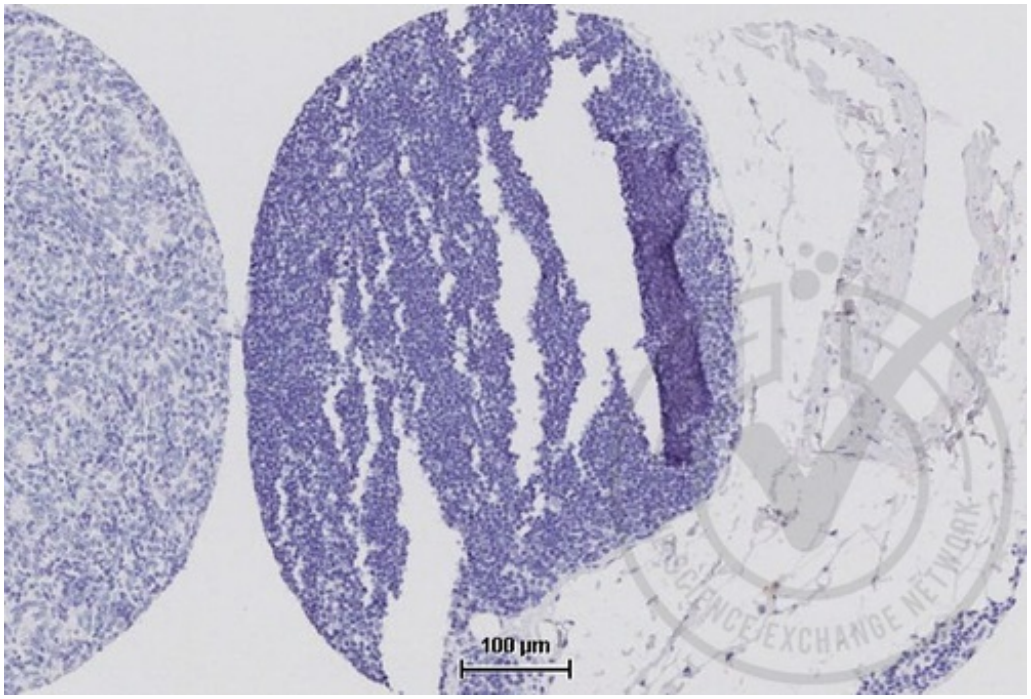

Figure 4: Isotype control immunostaining of human thymus (brown). Counterstain in blue.

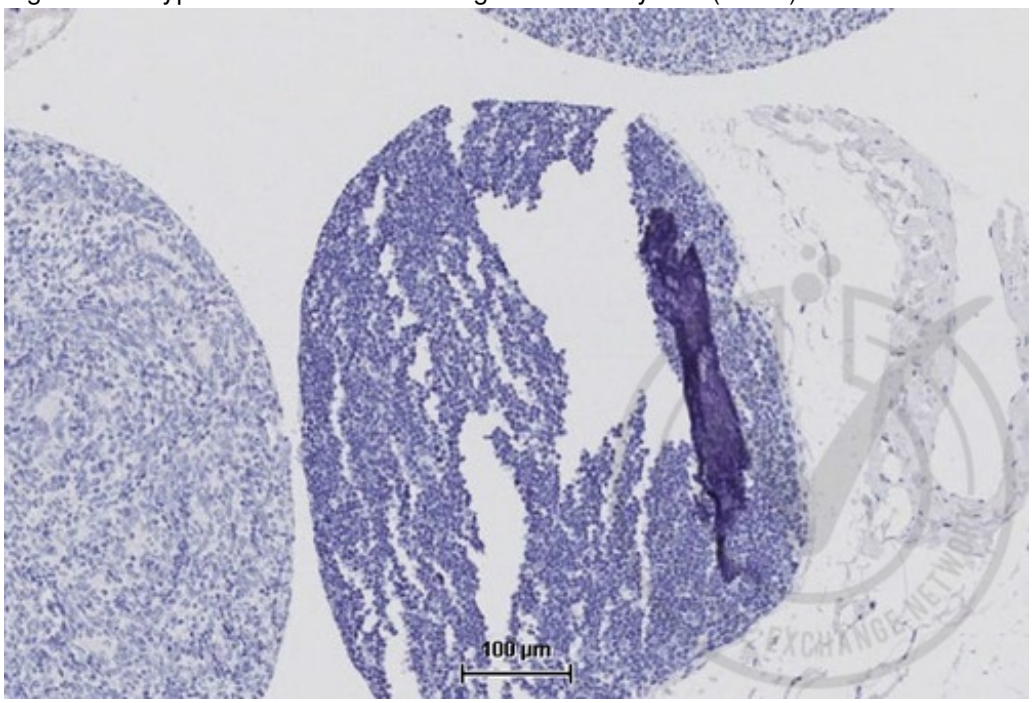

Figure 6: Secondary only immunostaining of human thymus (brown). Counterstain in blue.