Validation Report #029629

Validation Date: 03/16/14

Summary

Antigen	Wingless-Type MMTV Integration Site Family Member 2 (WNT2) (N-Term)
Catalog number	ABIN762896
Supplier	Bioss
Supplier catalog number	bs-6133R
Lot number	131105
Method validated	Immunohistochemistry
Laboratory	Histopathology and Tissue Shared Resource, Georgetown Lombardi Comprehensive Cancer Center
Validation number	029629
Positive Control	Human glioma tissue
Negative Control	Human brain
Notes	Signal was detected in positive control tissue and not in negative control tissue.

Full Methods

Primary Antibody
- Antigen: Wingless-Type MMTV Integration Site Family Member 2 (WNT2) (N-Term)
- Catalog number: ABIN762896
- Supplier: Bioss
- Supplier catalog number: bs-6133R
- Lot number: 131105

Isotype Control Antibody
- Antibody: Rabbit IgG isotype control
- Supplier: Immunoreagents
- Catalog number: Rb-003-N
- Batch number: 27-165-120613

Secondary Antibody
- Antibody: Envision Plus Horse Radish Peroxidase conjugated anti-rabbit antibody
- Catalog number: K4003
- Supplier: DAKO
- Batch number: 10082183

Controls
- Positive control: Human Glioma (specimen known to contain the target protein) from HTSR's Human Tissue Bank.
- Negative Control: Human Normal Brain (specimen known to not contain the target protein) from HTSR's Human Tissue Bank.
- Primary antibody isotype control: Human Glioma treated with primary antibody isotype control instead of the primary antibody.
- Secondary antibody only control: Human Glioma treated with secondary antibody only (no primary antibody).

Protocol

Immunohistochemistry was performed by hand.
- Sections were de-paraffinized on a Leica Autostainer in Xylenes (1X 5 min, 2X 2 min), and rehydrated through Ethanols (2X 100% for 5 min each, 95% 2X 2 min each, 80%, 70%) to running tap water.
- Sections were heated to 98°C for 20 min in 10 mM Sodium Citrate buffer pH 6.0 for antigen retrieval, then moved to RT in the same buffer for 20 min.
- Sections were rinsed in de-ionized water for 5 min at RT.
- Sections were blocked in Hydrogen Peroxide (Fisher, H325-500) for 30 min at RT.
- Sections were rinsed in Tris Buffered Saline with 0.5% Tween-20 (TBST) 2 times for 5 min at RT.
- Sections were blocked in 5% Normal Goat Serum for 2 h at RT.
- Sections were incubated with primary antibody diluted 1:250 in TBST overnight at 4°C.
- Sections were rinsed in TBST for 5 min 2X at RT.
- Sections were incubated with Envision Plus anti-Rabbit-Horse Radish Peroxidase conjugated Polymer for 2 h at RT.
- Sections were rinsed in TBST for 5 min 2X at RT.
- Sections were incubated with DAB chromogenic substrate (DAKO, K348) for 10 min at RT.
- Sections were washed x 1 in Distilled Water.
- Sections were counterstained with 1:9 dilution of Harris Hematoxylin (Fisher, SH30-500D) for 2 min.
- Sections were washed x 1 in Distilled Water.
- Sections were blued in Ammonium Hydroxide for 1 min.
- Sections were washed x 1 in Distilled Water.
- Sections were dehydrated through graded alcohols, mounted in Acrymount and photographed on an Olympus DX61 microscope with DP70 camera using DP Controller and DP Manager Software.

Experimental Notes
- The company recommends between 1:100 – 1:500 dilution factor; in our hands, 1:250 was overstained and we would recommend using a lower concentration of the antibody.
- The "normal" brain section we used proved to be from an individual who had morphological features of Parkinson's Disease. The area shown in Figure 2 is from the morphologically normal part of the brain. The area with Parkinson's morphology reacted strongly with the anti-wnt2 antibody (Figure 5).

Figures

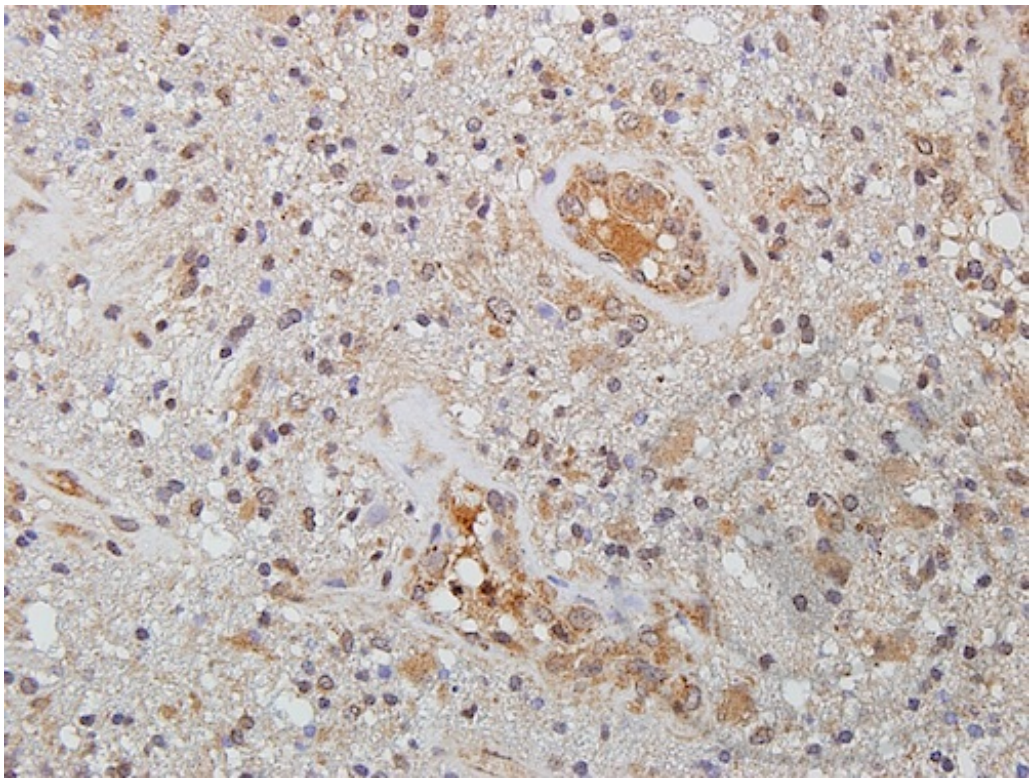

Figure 1: Human brain glioma tissue stained with anti-Wnt2 (brown) and counterstained with hematoxylin (blue).

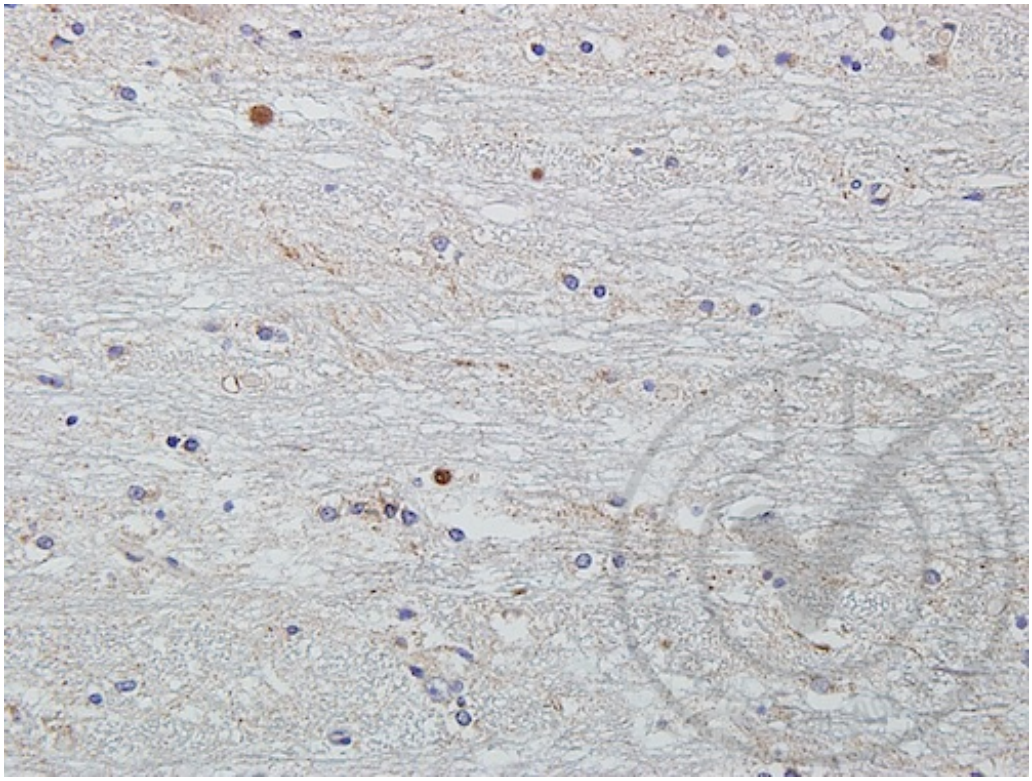

Figure 2: Human normal brain tissue stained with anti-Wnt2 (brown) and counterstained with hematoxylin (blue).

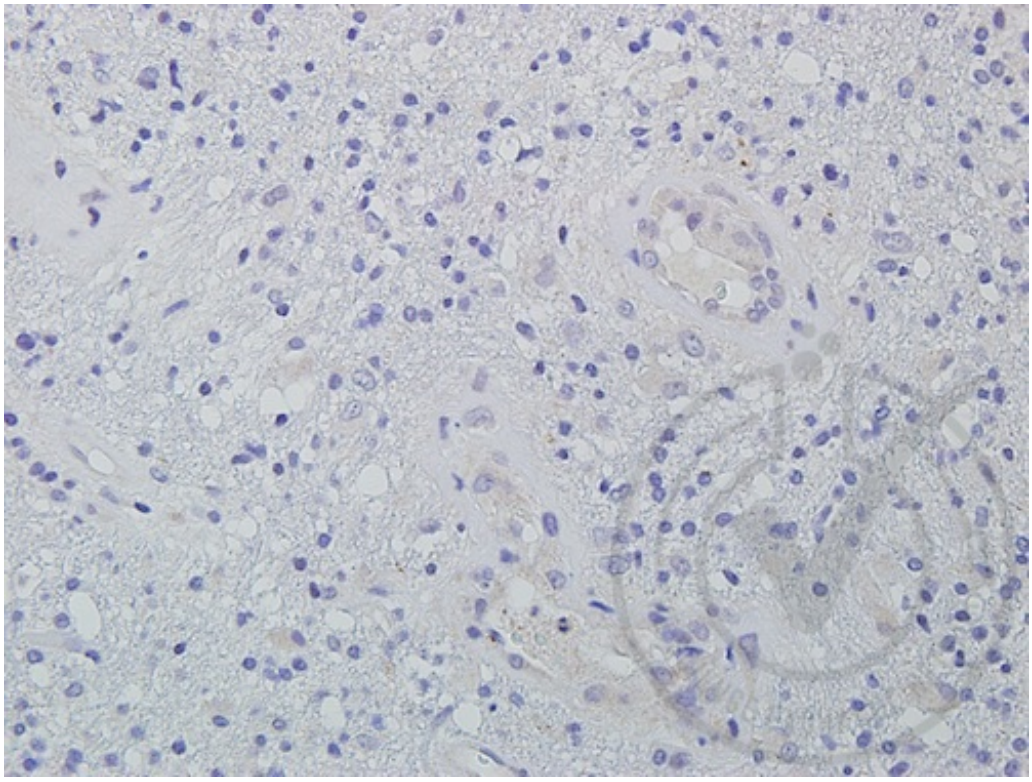

Figure 3: Human brain glioma tissue stained with isotype control antibody (brown) and counterstained with hematoxylin (blue).

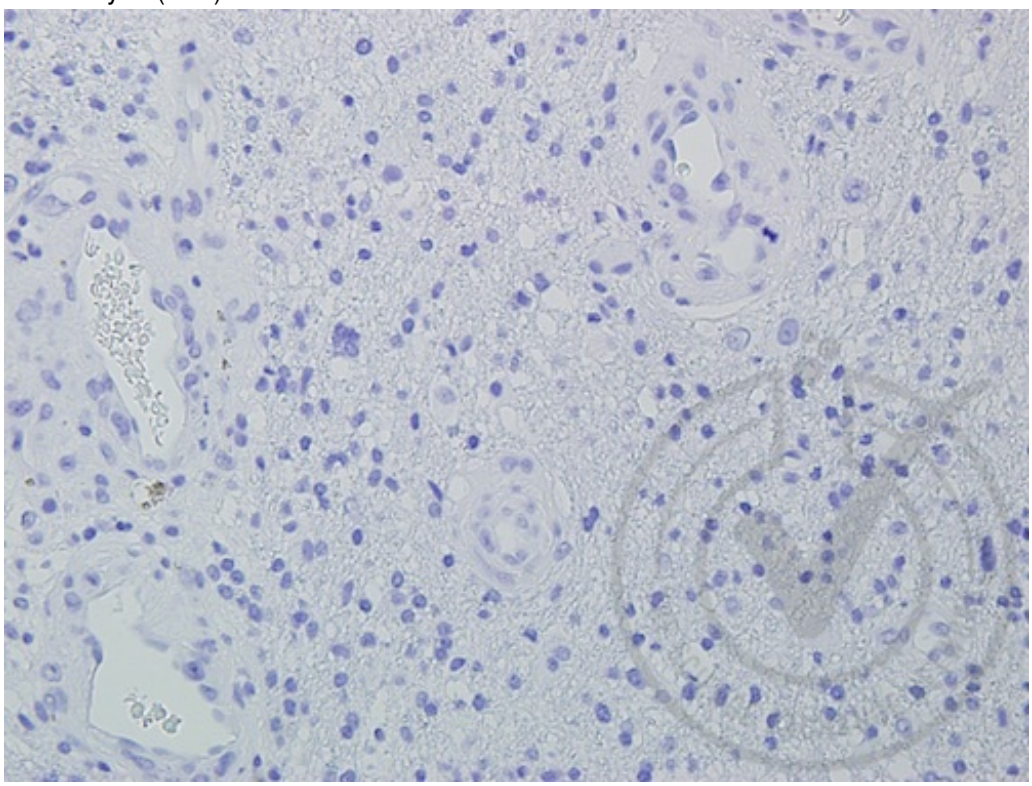

Figure 4: Human brain glioma tissue stained with secondary only (brown) and counterstained with hematoxylin (blue).

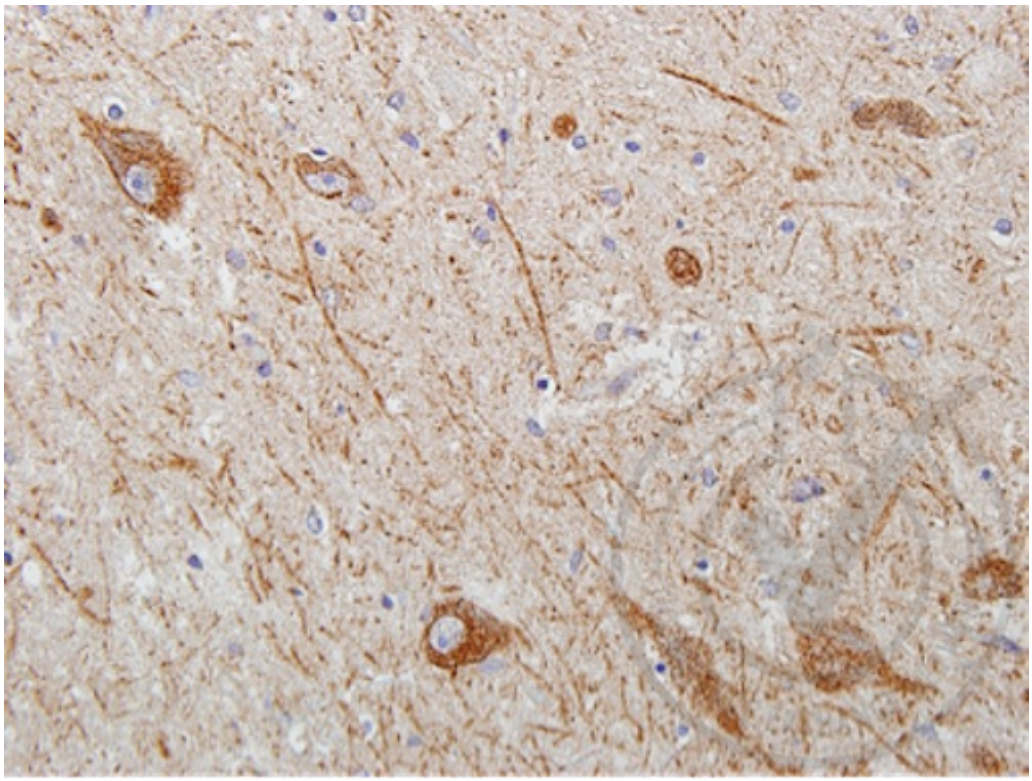

Figure 5: Human brain tissue with Parkinson's morphology stained with anti-Wnt2 (brown) and counterstained with hematoxylin (blue).