

Validation Report #029632

Validation Date: 03/23/14

Summary

Antigen	Occludin (OCLN)
Catalog number	ABIN687337
Supplier	Bioss
Supplier catalog number	bs-1495r
Lot number	990698W
Method validated	Immunofluorescence
Laboratory	Confocal Imaging Core, Beth Israel Deaconess Medical Center
Validation number	029632
Positive Control	Mouse stomach tissue
Negative Control	Human fat tissue
Notes	Signal was detected in positive control sample and not in negative control sample.



Full Methods

Primary Antibody

- Antibody: Occludin (OCLN)
- Catalog number: ABIN687337
- Supplier: Bioss
- Supplier catalog number: bs-1495r
- Lot number: 990698W

Isotype Control Antibody

- Antibody: Rabbit IgG
- Supplier: Bioss
- Catalog number: bs-0295p

Secondary Antibody

- Antibody: Donkey anti-Rabbit IgG (Heavy & Light Chain) Antibody (Alexa 647)
- Supplier: Jackson Immuno Research
- Catalog number: 711-605-152

Controls

- Positive control: PPFE mouse stomach tissue
- Negative Control: PPFE human fat tissue
- Isotype antibody control: tissue sections treated with Rabbit IgG Isotype at 1ug/ul , Bioss bs-0295p
- Secondary antibody only control: positive and negative control tissue sections treated with Goat anti-Rabbit Alexa 647 secondary antibody only.

Protocol

- Paraffin embedded positive and negative control tissue sections were deparaffinized and undergone antigen retrieval using 10 mM Sodium Citrate pH6 in a pressure cooker for 10 min.
- The tissue sections were incubated with 1 mg/mL Sodium borohydride for 10 min at room temperature to block autofluorescent background signal. The sections were then rinsed three times in TBS for 5 min each at RT.
- Tissue sections were blocked in 1 X TBS / 5% normal donkey serum for 60 min at RT.
- Tissue sections were incubated with primary antibody diluted 1:200 in 1X PBS / 5% normal donkey serum overnight at 4°C.
- Tissue sections were rinsed three times in TBS for 5 min each at RT.
- Cells were incubated with secondary antibody diluted 1:200 in 1X PBS / 5% normal donkey serum for 60 min at RT in dark.
- Tissue sections were rinsed three times in TBS for 5 min each at RT.
- Coverslips were mounted on slides with Prolong Gold anti-fade mounting media (Invitrogen)
- IF stained tissue sections were imaged with a Zeiss LSM 510 Meta confocal microscope.

Experimental Notes

- No challenges notes
- OCLN is recommended to staining of mouse stomach tissue according to this protocol.

Figures

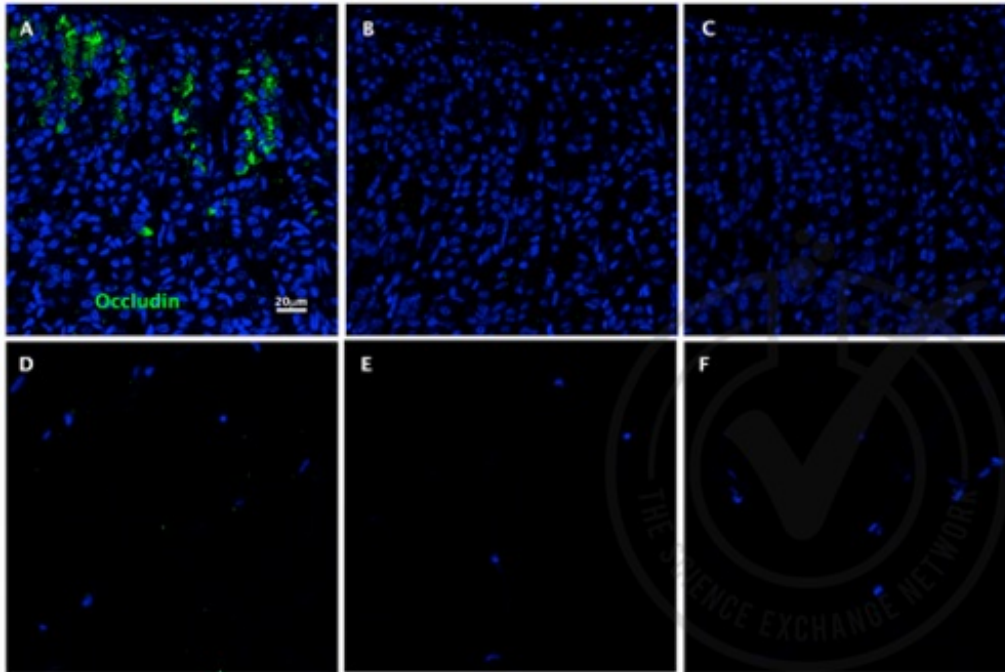


Figure 1: Immunostaining (green) of mouse stomach tissue (A-C) and human fat tissue (D-F) with anti-OCLN (A, D), isotype control antibody (B, E), or with secondary alone (C, F). Tissues were counterstained with DAPI (blue).