Validation Report #029613

Validation Date: 02/20/14

Summary

Antigen	Allograft Inflammatory Factor 1 (AIF1)
Catalog number	ABIN685477
Supplier	Bioss
Supplier catalog number	bs-1363r
Lot number	980993W
Method validated	Immunofluorescence
Laboratory	Reveal Biosciences
Validation number	29613
Positive Control	Mouse spleen
Negative Control	Mouse duodenum
Notes	Strong signal was observed in the positive control tissue, while no signal was observed in the negative control tissue.

Full Methods

Primary Antibody
- Antibody: AIF1
- Catalog number: ABIN685477
- Supplier: Bioss
- Supplier catalog number: bs-1363r
- Lot number: 980993W

Isotype Control Antibody
- Antibody: Rabbit IgG isotype control
- Catalog number: I5006
- Supplier: Sigma
- Batch number: SLBD3695V

Secondary Antibody
- Antibody: Alexa Fluor 546 Donkey Anti Rabbit
- Catalog number: A10040
- Supplier: Life Technologies
- Lot number: 1218269

Additional Information

Controls
- Positive control: Wild type mouse spleen (specimen known to contain the target protein) from Explora BioLabs.
- Negative Control: Wild type mouse duodenum (specimen known to not contain the target protein) from Explora BioLabs.
- Primary antibody isotype control: Wild type mouse spleen treated with primary antibody isotype control instead of the primary antibody.
- Secondary antibody only control: Wild type mouse spleen treated with secondary antibody only (no primary antibody).

Protocol
- Frozen, OCT embedded tissues were cut 5 µm and mounted on positive charged slides.
- Sections were air dried for 30 min prior to fix in 10% neutral buffered formalin for 10 min.
- Fixed slides were rinsed three times in PBS for 5 min each at RT.
- Sections were blocked in 1 X PBS / 3% donkey serum / 0.1% Triton X-100 for 10 min at RT.
- Sections were incubated with primary antibody diluted 1:100 in Universal Antibody Dilution Buffer (Electron Microscopy Sciences, 25886-05) overnight at 4°C.
- Sections were rinsed three times in PBS for 5 min each at RT.
- Sections were incubated with secondary antibody diluted 1:200 in Universal Antibody Dilution Buffer (Electron Microscopy Sciences, 25886-05) for 120 min at RT in dark.
- Sections were mounted on slides with FluoroGel II with DAPI (Electron Microscopy Sciences).
- Stained sections were imaged with a 3D Histech Pannoramic SCAN 150.

Experimental Notes

None

Figures

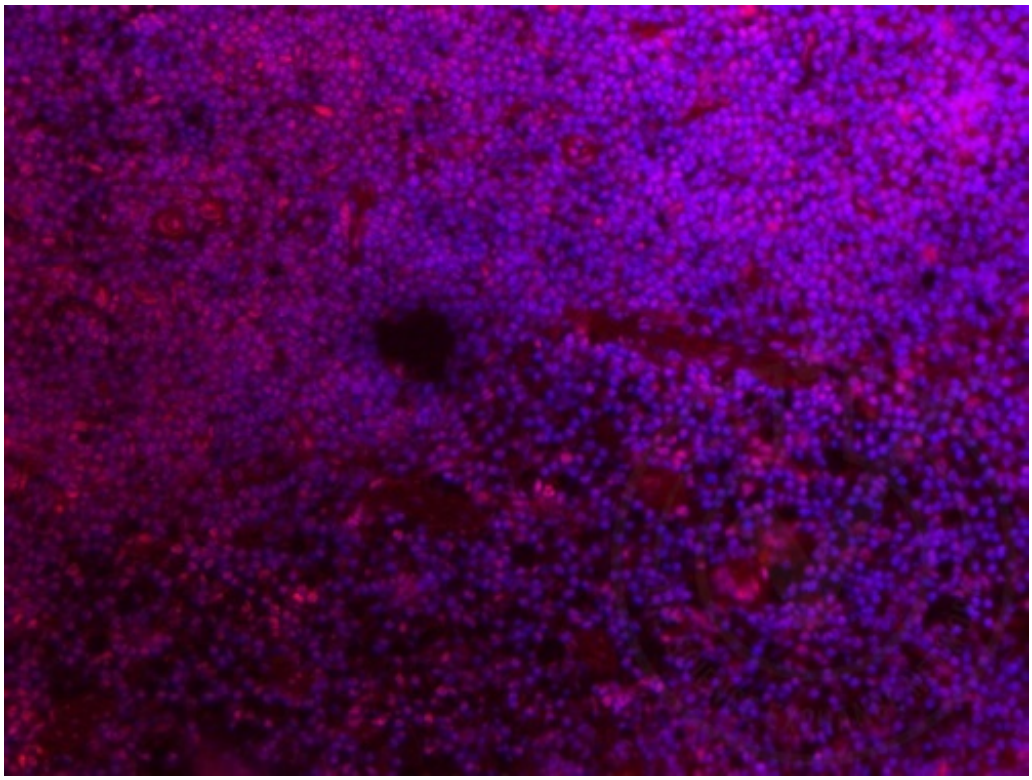

Figure 1: AIF1 staining on mouse spleen (red), counterstained with DAPI (blue).

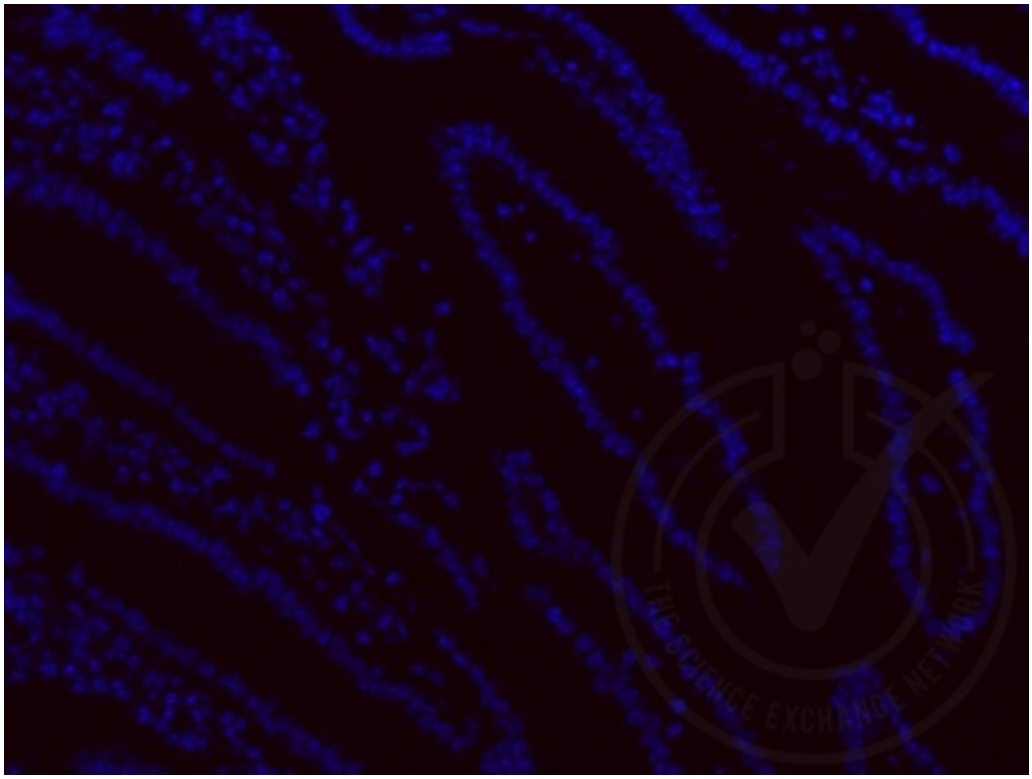

Figure 2: AIF1 staining on mouse duodenum (red), counterstained with DAPI (blue).

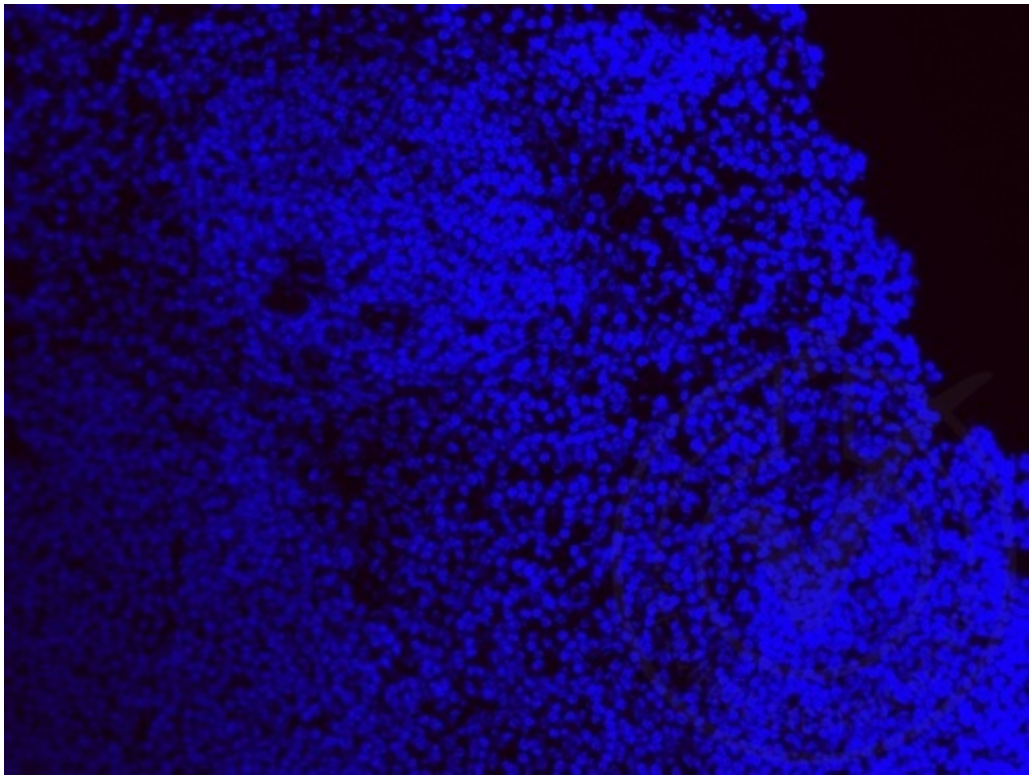

Figure 3: Isotype control staining on mouse spleen (red), counterstained with DAPI (blue).

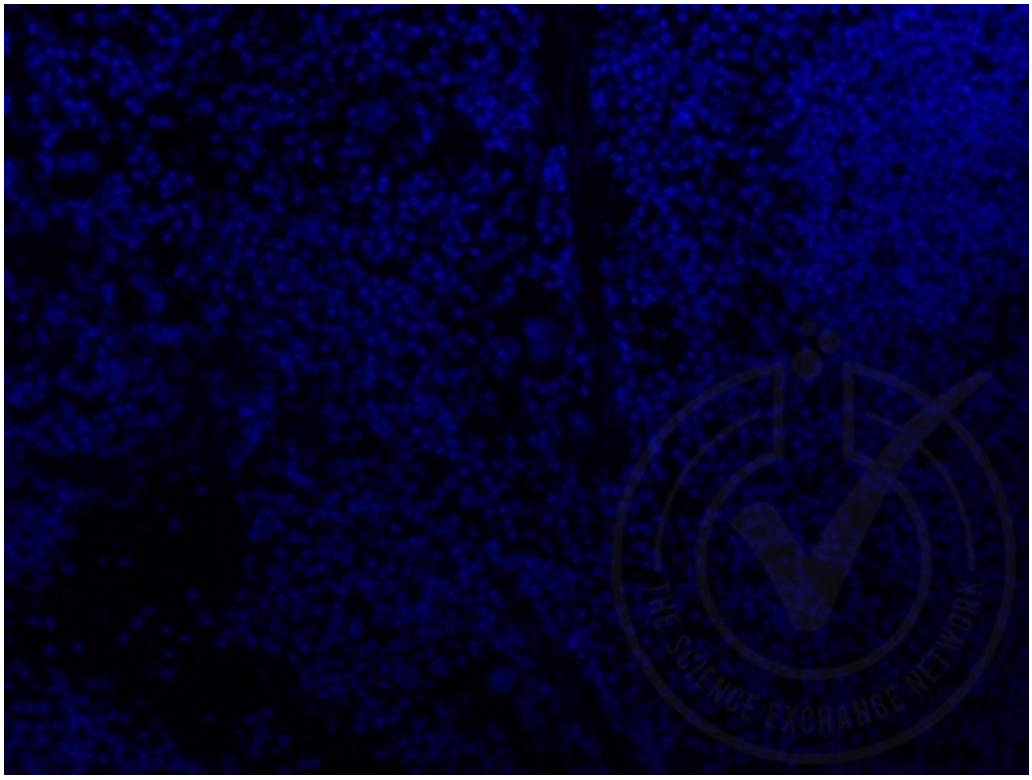

Figure 4: Secondary only staining on mouse spleen (red), counterstained with DAPI (blue).