# Validation Report #029732

Validation Date: 06/11/14

## Summary

| | |
|---|---|
| Antigen | Tumor Necrosis Factor (TNF) |
| Catalog number | ABIN677318 |
| Supplier | Bioss |
| Supplier catalog number | bs-2081R |
| Lot number | 140113 |
| Method validated | Immunofluorescence |
| Laboratory | Confocal Imaging Core, Beth Israel Deaconess Medical Center |
| Validation number | 29732 |
| Positive Control | Normal mouse lung tissue |
| Negative Control | Normal mouse adipose tissue |
| Notes | Fluorescent signal is detected in positive control, but not in negative control or isotype controls. |

# Full Methods

**Primary Antibody**
- Antibody: Tumor Necrosis Factor (TNF)
- Catalog number: ABIN677318
- Supplier: Bioss
- Supplier catalog number: bs-2081R
- Lot number: 140113

**Isotype Control Antibody**
- Antibody: Rabbit IgG Isotype
- Supplier: Bioss
- Supplier catalog number: bs-0295p
- Lot number: not available

**Secondary Antibody**
- Antibody: Donkey anti-Rabbit IgG (Heavy & Light Chain) Antibody (Alexa 647)
- Catalog number: 711-605-152
- Supplier: Jackson Immuno Research
- Lot number: Not available

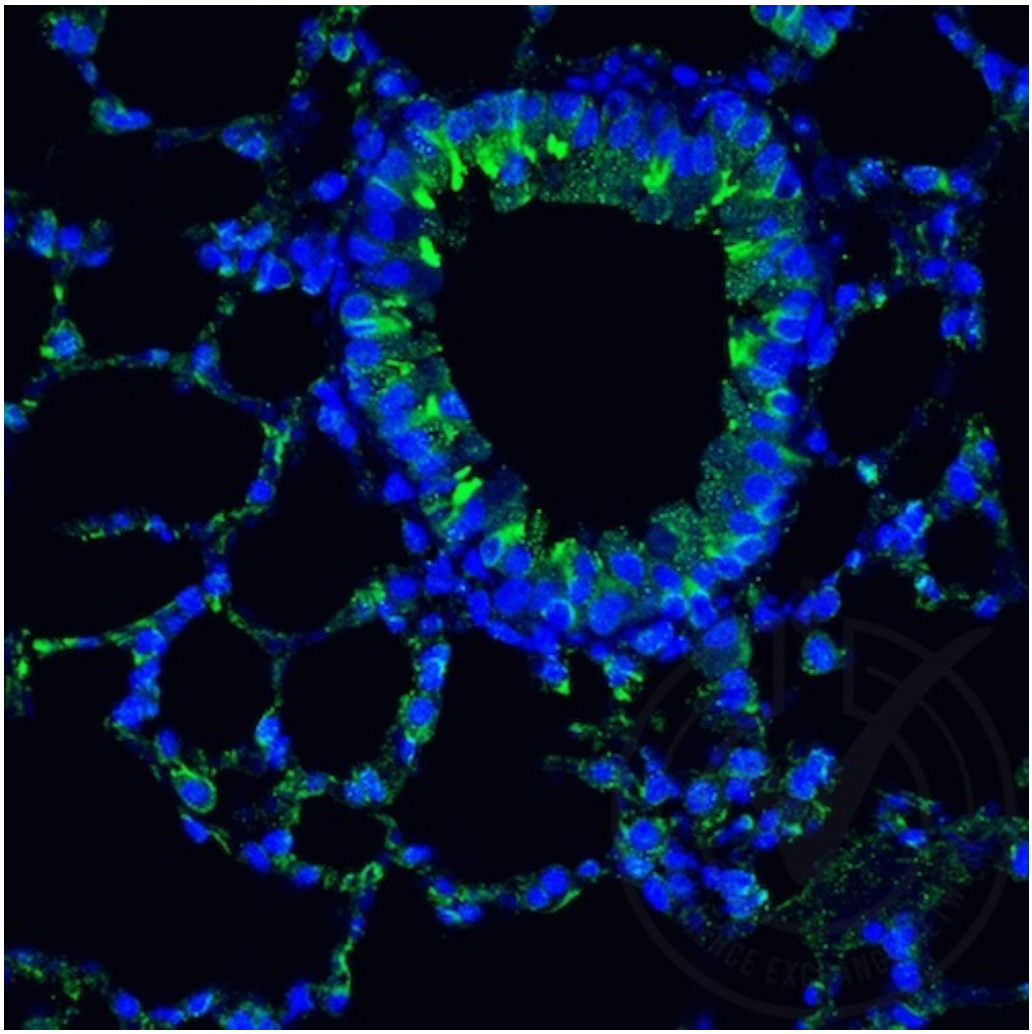
**Controls**
- Positive control: FFPE normal mouse lung tissue
- Negative Control: FFPE normal mouse fat tissue
- Isotype antibody control: tissue sections treated with Rabbit IgG Isotype at 1ug/ul
- Secondary antibody only control: positive and negative control tissue sections treated with Goat anti-Rabbit Alexa 647 secondary antibody only. Any staining observed is due to non-specific binding of secondary antibody.

**Protocol**
- Paraffin embedded positive and negative control tissue sections were deparaffinized and underwent antigen retrieval using 10 mM sodium citrate pH 6.0 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.

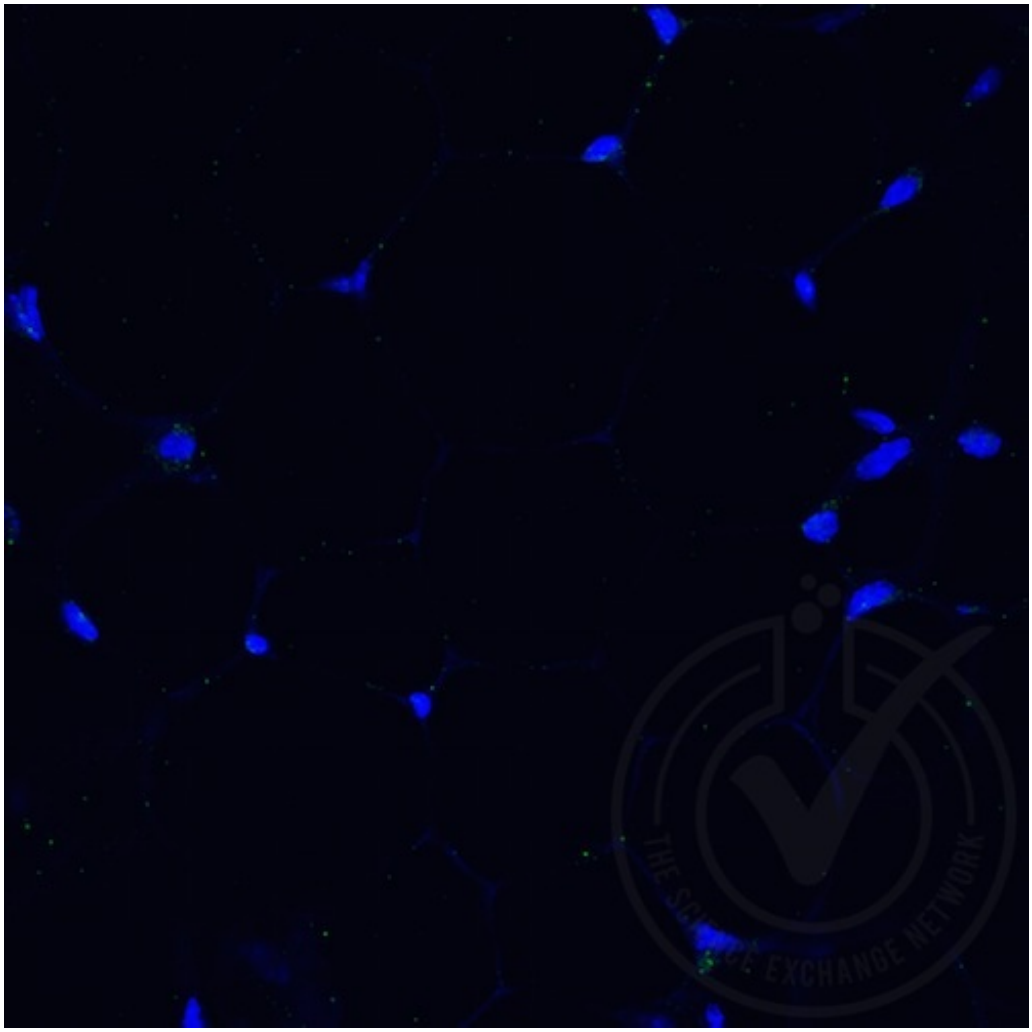
**Experimental Notes**

Nothing to note.

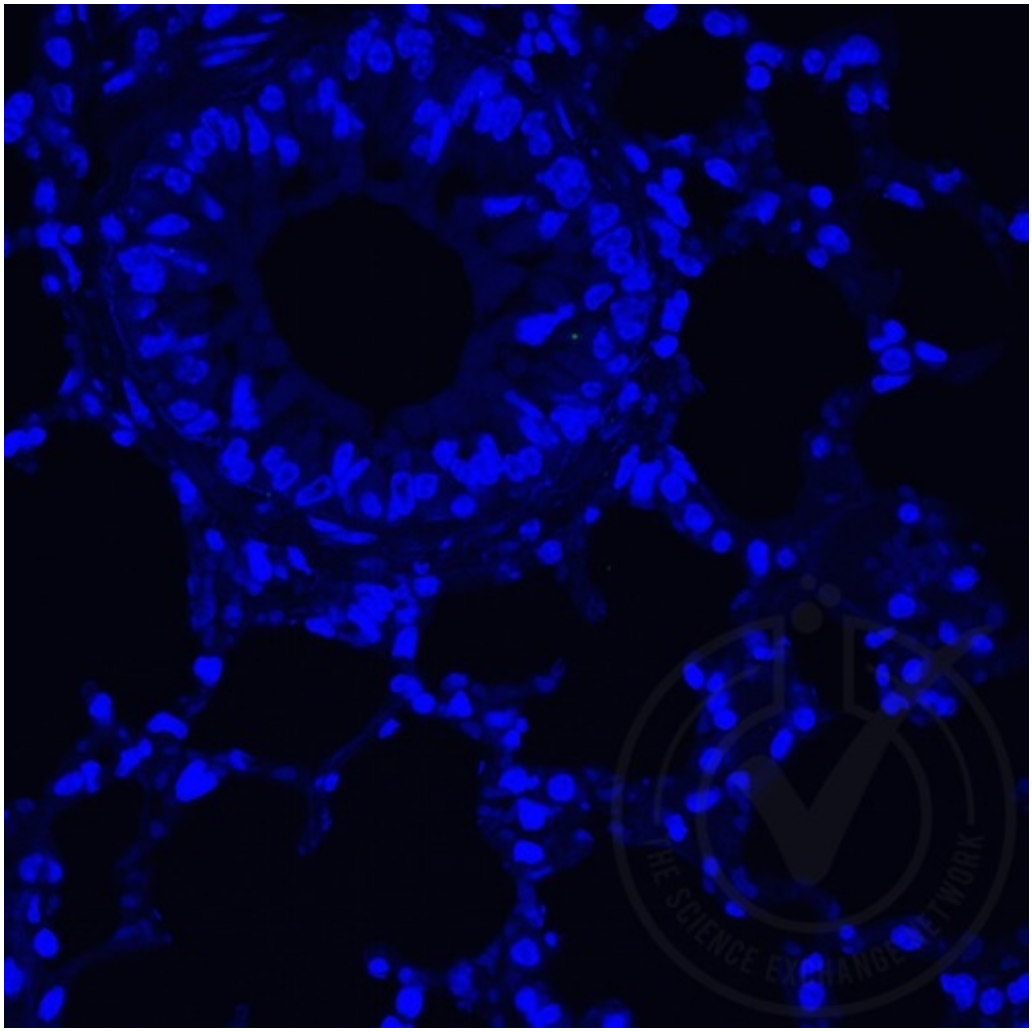
## Figures



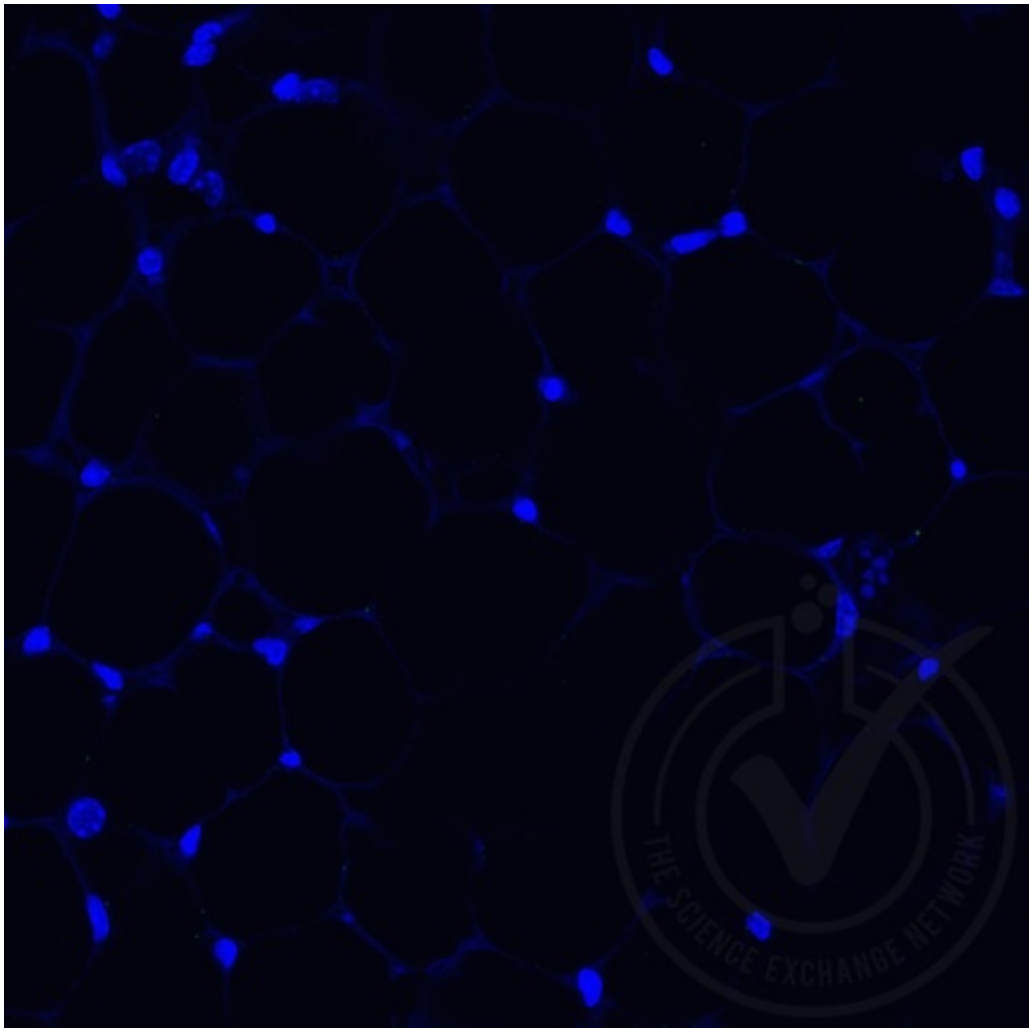
Panel 1: confocal image of positive control (mouse lung) with anti-TNF alpha antibody



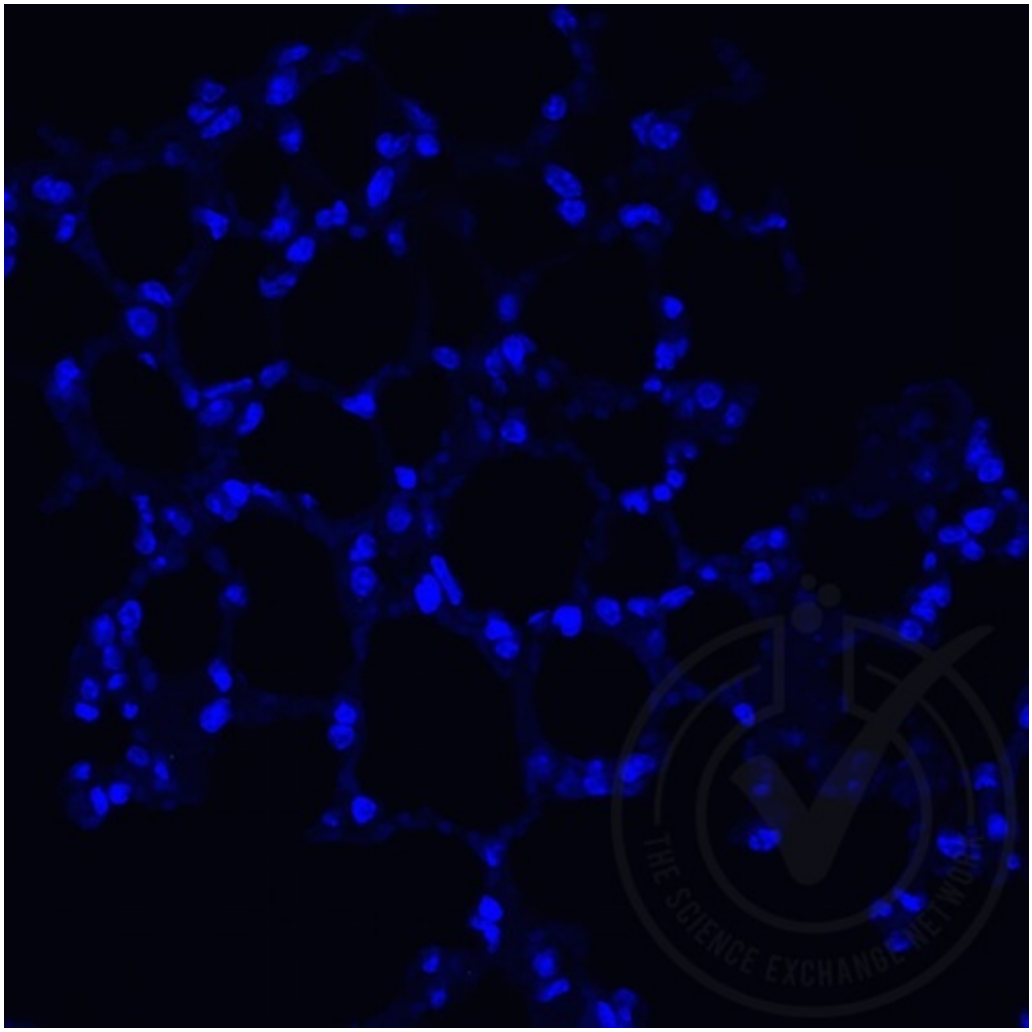
Panel 2: confocal image of negative control (mouse adipose) with anti-TNF alpha antibody



Panel 3: confocal image of positive control (mouse lung) with isotype control



Panel 4: confocal image of negative control (mouse adipose) with isotype control



Panel 5: confocal image of positive control (mouse lung) with secondary antibody only