

Validation Report #029683

Validation Date: 04/25/14

Summary

Antigen	Mdm2, p53 E3 Ubiquitin Protein Ligase Homolog (Mouse) (MDM2)
Catalog number	ABIN736821
Supplier	Bioss
Supplier catalog number	bs-1043R
Lot number	110602
Method validated	Immunofluorescence
Laboratory	Molecular Pathology Core, University of Florida
Validation number	029683
Positive Control	Human breast carcinoma
Negative Control	Pig muscle tissue (non-reactive species)
Notes	Signal was detected in positive control sample and not in negative control sample. Note low levels of background in negative control and isotype control samples.



Full Methods

Primary Antibody

- Antigen: Mdm2, p53 E3 Ubiquitin Protein Ligase Homolog (Mouse) (MDM2)
- Catalog number: ABIN736821
- Supplier: Bioss
- Supplier catalog number: bs-1043R
- Lot number: 110602

Isotype Control Antibody

- Antibody: Rabbit IgG control
- Supplier: Vector
- Catalog number: I-1000
- Lot number: T0503

Secondary Antibody

- Antibody: AF 488 goat anti-rabbit IgG
- Supplier: Invitrogen
- Catalog number: A11034
- Lot number: 702323

Controls

- Positive control: FFPE human breast carcinoma block from Molecular Pathology Core.
- Negative Control: FFPE Pig skeletal muscle block from Molecular Pathology Core.
- Secondary antibody only control: Human breast carcinoma treated with Goat anti-Rabbit AF488 secondary antibody only.
- Isotype control: FFPE human Breast Carcinoma block from Molecular Pathology Core.

Protocol

- Human breast carcinoma and pig skeletal muscle (formalin fixed paraffin embedded) blocks were from Molecular Pathology Core.
- The 4 μ m thick sections were treated by Citra in steamer for 20 min and cool down on the bench for another 20 min.
- Sections were blocked in 1 X TBS / 5% normal goat serum for 2 h at RT.
- Sections were incubated with primary antibody diluted 1:100 in 1xTBS overnight at 4°C.
- Sections were rinsed three times in TBS for 5 min each at RT.
- Sections were incubated with secondary antibody diluted 1:1000 in 1xTBS for 60 min at RT in dark.
- Sections were rinsed three times in TBS for 5 min each at RT.
- Coverslips were mounted on slides with DAPI (Invitrogen, Prolong Gold antifade reagent with DAPI)
- Stained sections were imaged with a Zeiss Axioskop2 microscope.

Experimental Notes

- MDM2 on pig tissues were negative and MDM2 on human breast CA tissues showed nuclei staining, although with some faint cytoplasmic staining.

Figures

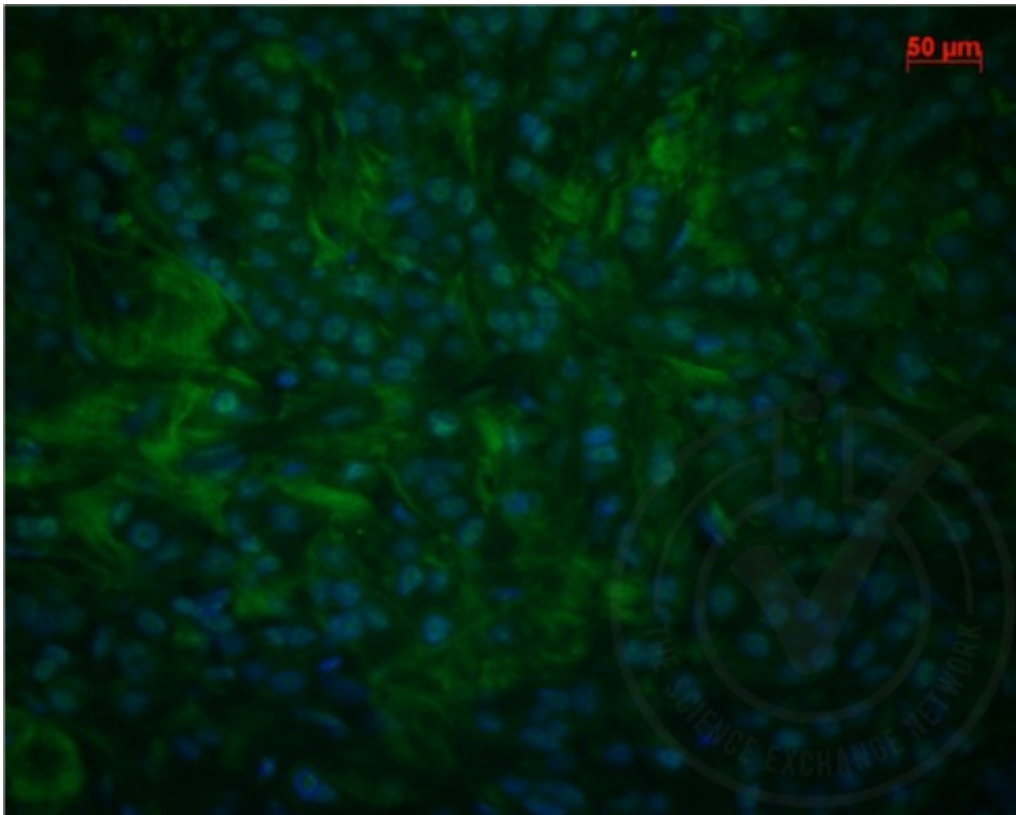


Figure 1: Human breast cancer tissue stained with anti-MDM2 (green) and counterstained with DAPI (blue).

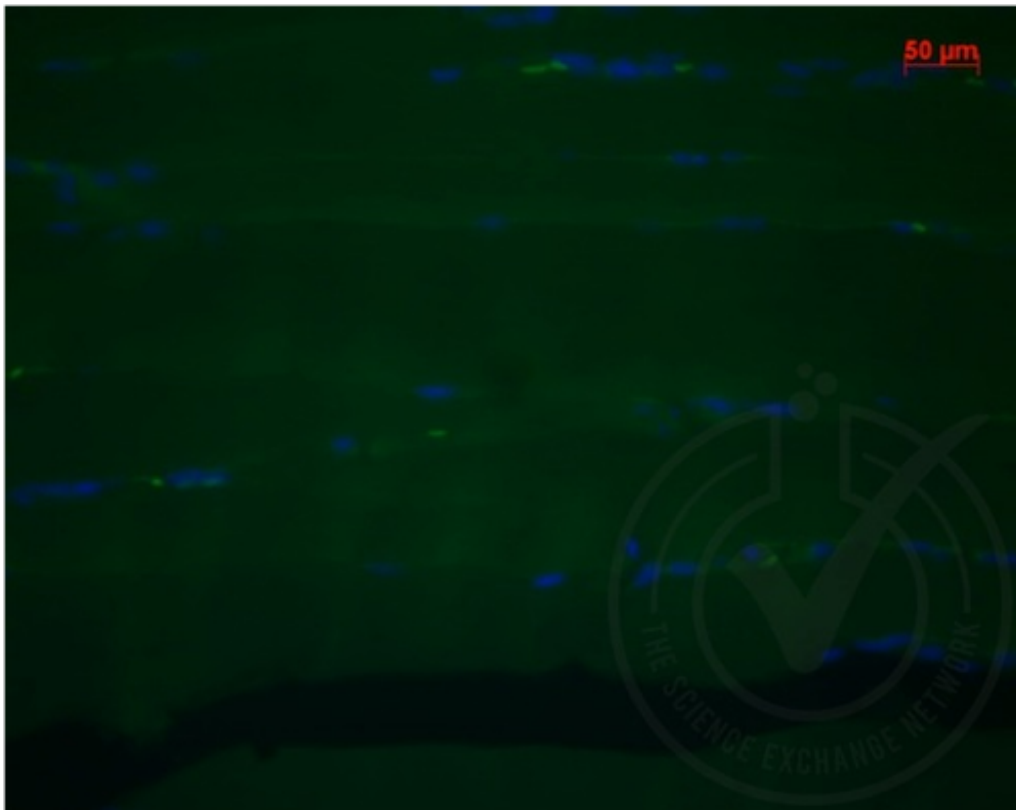


Figure 2: Pig muscle tissue stained with anti-MDM2 (green) and counterstained with DAPI (blue).

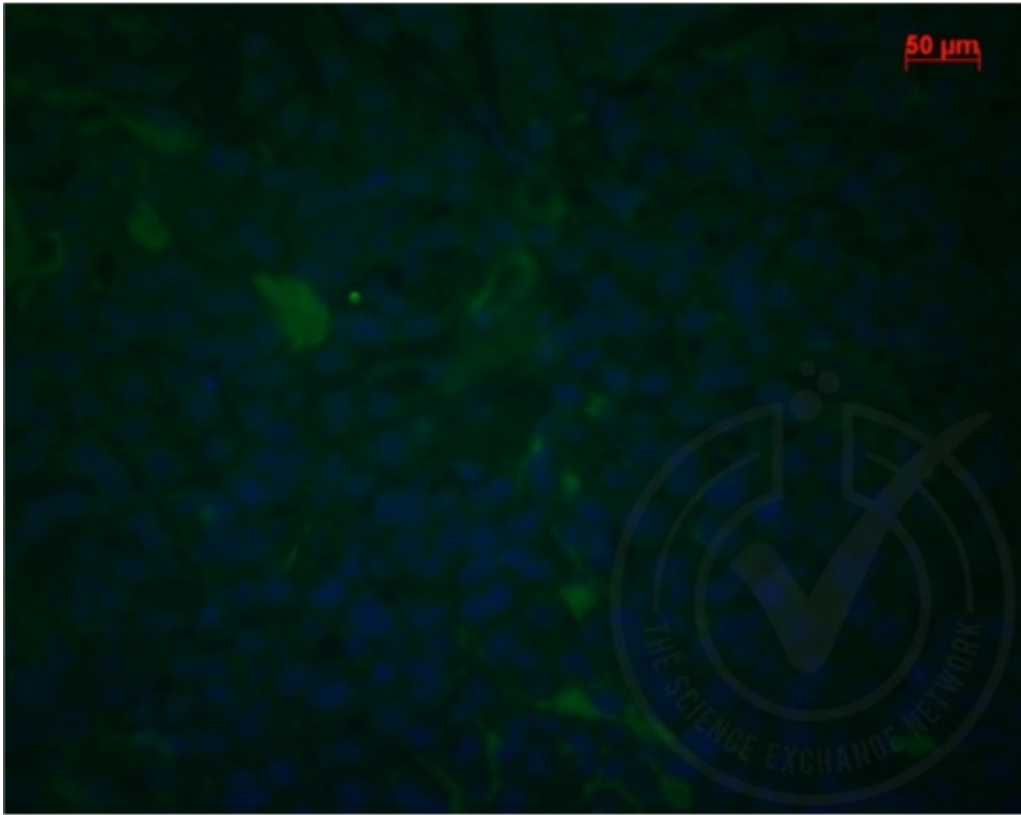


Figure 3: Human breast cancer tissue stained with isotype control antibody (green) and counterstained with DAPI (blue).

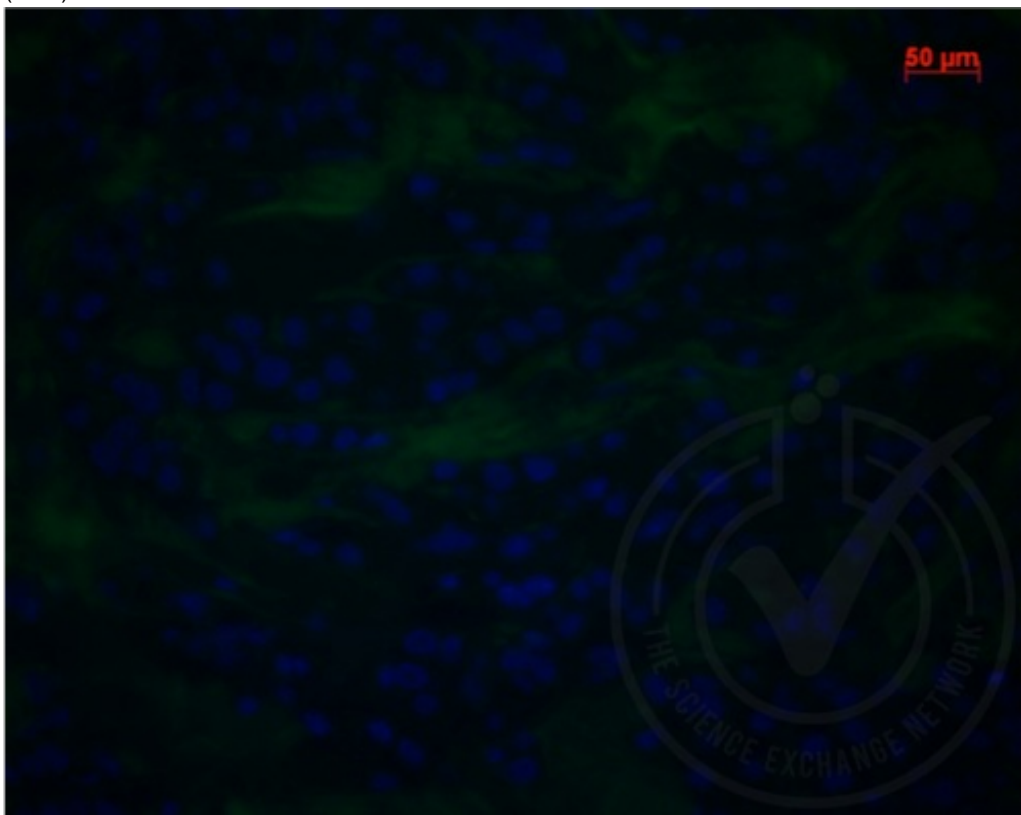


Figure 1: Human breast cancer tissue stained with secondary antibody only (green) and counterstained with DAPI (blue).