Validation Report #028751

Validation Date: 09/25/13

Summary

Antigen	Elastin (ELN) (C-Term)
Catalog number	ABIN734003
Lot number	120306
Method validated	Immunohistochemistry
Laboratory	Beth Israel Deaconess Medical Center Confocal Imaging Core
Supplier	Bioss
Supplier catalog number	bs-1756r
Validation number	28751
Positive Control	Mouse heart tissue
Negative Control	Mouse adipose tissue
Notes	Signal was detected in positive control tissue and not detected in negative control tissue.

Full Methods

Primary Antibody
- Antibody: Elastin (ELN) (C-Term) antibody
- Catalog number: ABIN734003
- Lot number: 120306

Isotype Control Antibody
- Antibody: Rabbit IgG isotype control
- Catalog number: bs-0295P
- Supplier: Bioss
- Lot number: YYDW72P

Secondary Antibody
- Antibody: Donkey anti-Rabbit IgG Antibody (Biotinylated)
- Catalog number: 711-065-152
- Supplier: Jackson ImmunoResearch

Controls
- Positive control: Wild type mouse heart tissue (specimen known to contain the target protein).
- Negative control: Wild type adipose tissue (specimen known to not contain the target protein).
- Primary antibody isotype control: Wild type mouse heart treated with primary antibody isotype control instead of the primary antibody.
- Secondary antibody only control: Wild type mouse heart treated with secondary antibody only (no primary antibody).

Protocol

Immunohistochemistry was performed manually.
- Sections were deparaffinized and rehydrated.
- Sections were heated to 98°C for 10 min in 10 mM citrate buffer pH 6.0 for antigen retrieval.
- Sections were incubated in 3% hydrogen peroxide for 10 min at room temperature to block endogenous peroxidase.
- Sections were washed x 3 in Tris buffered saline (TBS).
- Sections were blocked in 5% normal donkey serum for 60 min at room temperature.
- Sections were incubated with primary antibody diluted 1:1000 in 5% normal donkey serum in TBS. Incubated at 4°C overnight.
- Sections were washed x 3 in Tris buffered saline.
- Sections were incubated with secondary antibody diluted 1:400 in 5% normal donkey serum in TBS. Incubated for 60 min at room temperature.
- Sections were washed x 3 in Tris buffered saline.
- Sections were incubated with Vectastain ABC kit (Vector Lab PK6100) to enhance signal.
- Sections were incubated with DAB chromogenic substrate (Vector Lab SK-4105) for 60 seconds at room temperature.
- Sections were washed x 3 in Distilled Water.
- Sections were counterstained with hematoxylin for 1 min.
- Sections were washed x 1 in Distilled Water.
- Sections were dehydrated, mounted and photographed under a Zeiss AxioImager M1 light microscope.

Experimental Notes

None

Figures

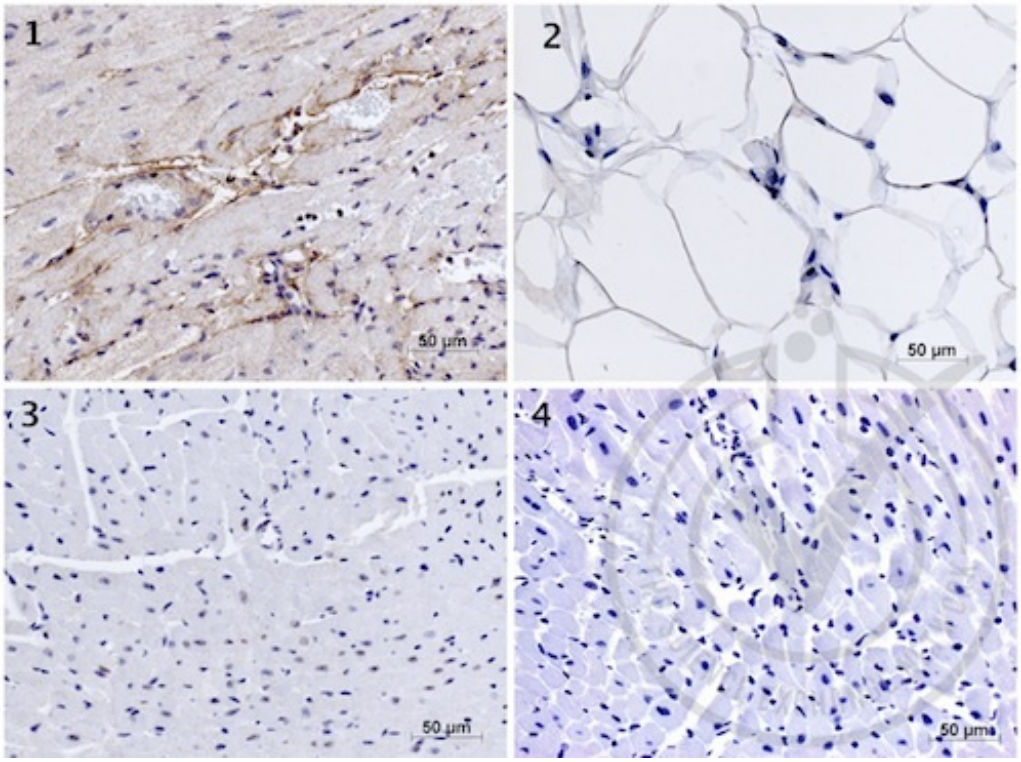

Figure 1: Immunostaining of Elastin antibody. Panel 1: Staining of mouse heart tissue stained with anti-Elastin antibody (brown). Panel 2: Staining of mouse adipose tissue with anti-Elastin antibody. Panel 3: Staining of mouse heart tissue with isotype control antibody. Panel 4: Staining of mouse heart tissue with secondary antibody only. All panels are counterstained with hematoxylin (blue).