Validation Report #029634

Validation Date: 03/26/14

Summary

Antigen	CD4 Molecule (CD4)
Catalog number	ABIN671376
Supplier	Bioss
Supplier catalog number	bs-0647R
Lot number	130703
Method validated	Immunohistochemistry
Laboratory	Confocal Imaging Core, Beth Israel Deaconess Medical Center
Validation number	029634
Positive Control	Mouse spleen tissue
Negative Control	Mouse muscle tissue
Notes	Signal was detected in positive control sample and not in negative control sample.

Full Methods

Primary Antibody
- Antibody: CD4 Molecule (CD4)
- Catalog number: ABIN671376
- Supplier: Bioss
- Supplier catalog number: bs-0647R
- Lot number: 130703

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

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

Controls
- Positive control: Spleen tissue (specimen known to contain the target protein) from mouse.
- Negative Control: MusIce tissue (specimen known to not contain the target protein) from mouse.
- Primary antibody isotype control: Mouse spleen treated with primary antibody isotype control instead of the primary antibody.
- Secondary antibody only control: Mouse spleen 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 0.1M Borate buffer with 1 mM NaCl and 1 mM EDTA pH 8.0 for antigen retrieval.
- Sections were incubated in 3% hydrogen peroxide for 10 min at room temperature to block endogenous peroxidases.
- 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 1:200 in 5% normal donkey serum in TBS. Incubated at 4°C temperature 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 sec at RT.
- Sections were washed x 3 in Distilled Water.
- Sections were counterstained with hematoxylin for 20 sec.
- Sections were washed x 1 in Distilled Water.
- Sections were dehydrated, mounted and photographed under a Zeiss AxioImager M1 light microscope.

Experimental Notes
- No experimental challenges noted.

Figures

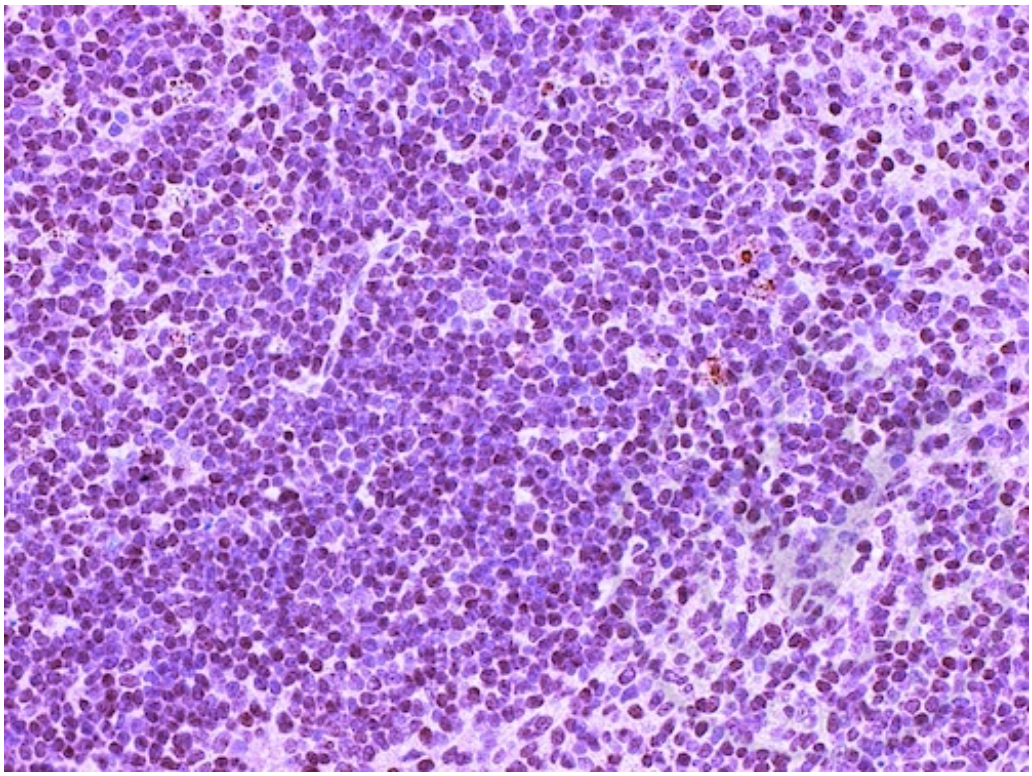

Figure 1: Mouse spleen stained with anti-CD4 (brown) and counterstained with hematoxylin.

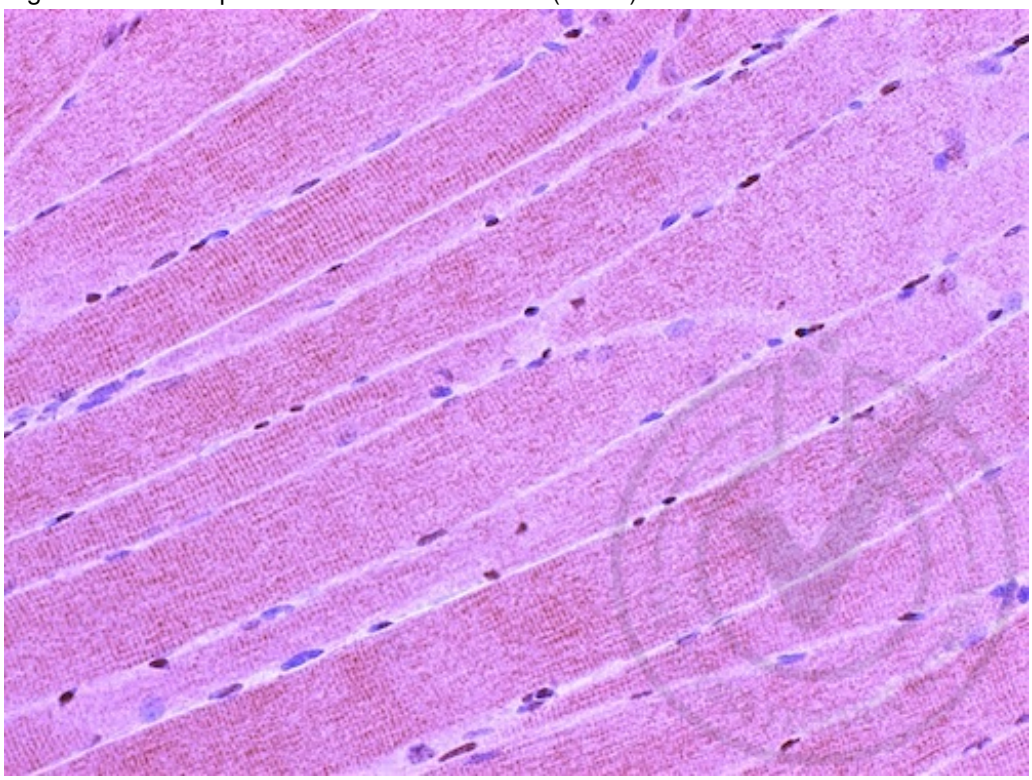

Figure 2: Mouse muscle stained with anti-CD4 (brown) and counterstained with hematoxylin.

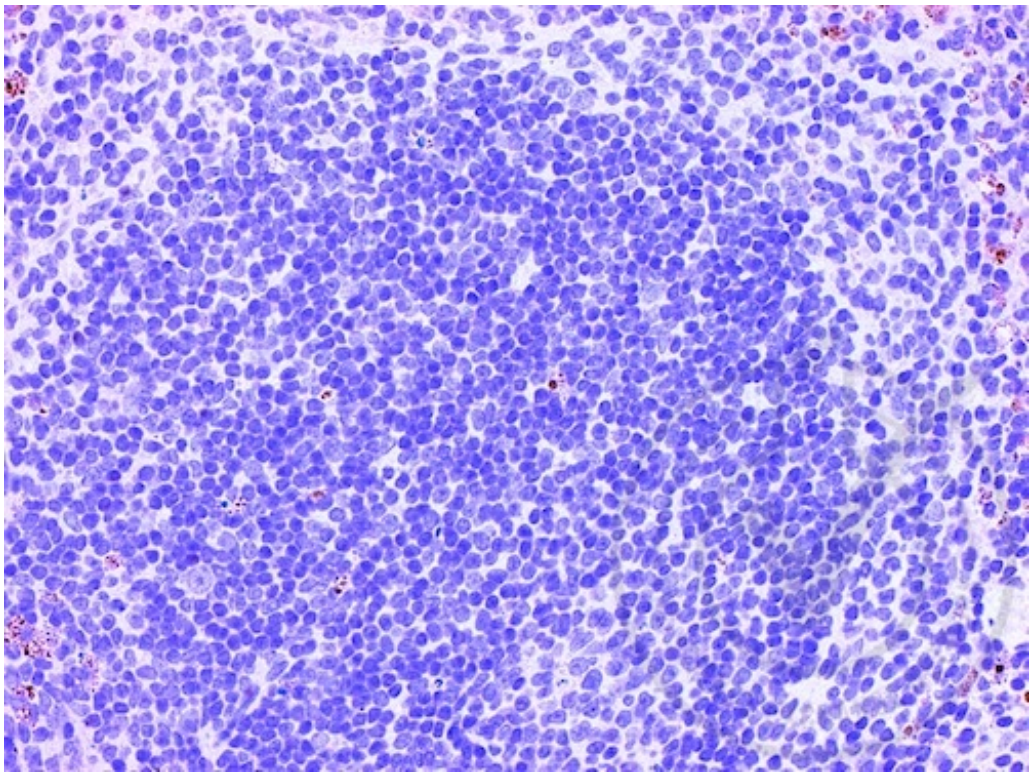

Figure 3: Mouse spleen stained with isotype control antibody (brown) and counterstained with hematoxylin.

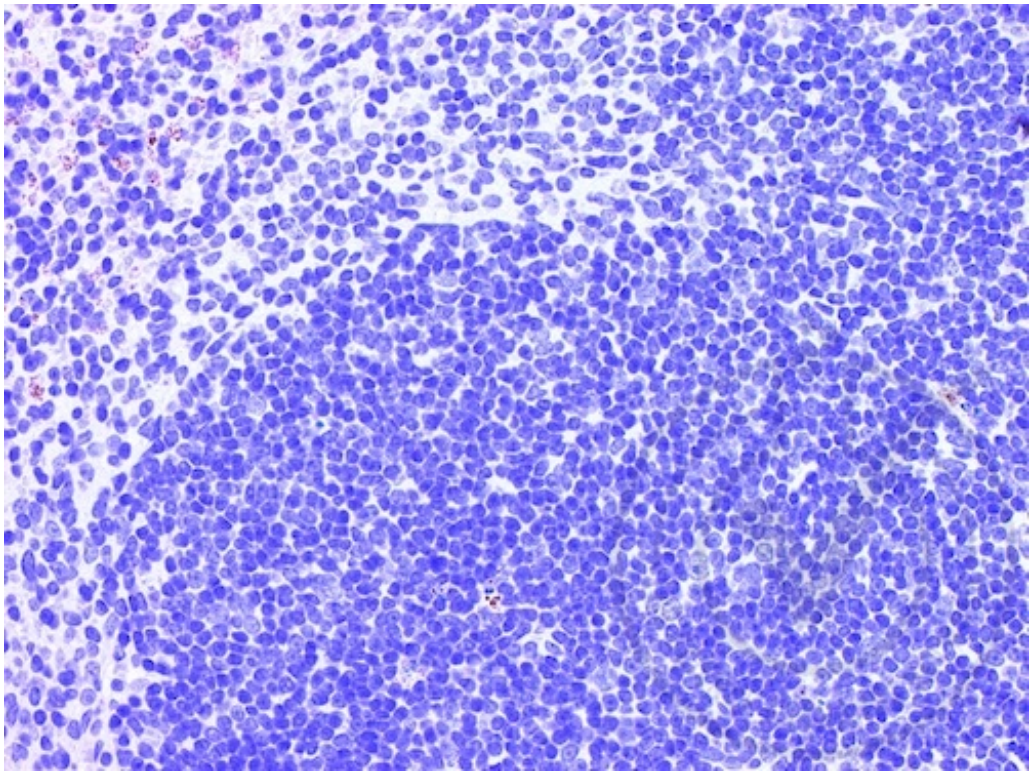

Figure 4: Mouse spleen stained with secondary antibody only (brown) and counterstained with hematoxylin.