

MDC1A\_M.1.2.005

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## **Inflammation quantification in the LAMA2<sup>dyW</sup> mouse**

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Authors	Jinger Doe & Dean Burkin University of Nevada Reno, School of Medicine
Working group members	Janice Dominov (University of Massachusetts Medical School, Worcester, MA, USA)
SOP responsible	Dean Burkin
Official reviewer	Janice Dominov

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## 1. OBJECTIVE

The objective of this SOP is to provide a detailed description of quantifying inflammation in the  $dy^W$  mouse model of MDC1A by immunohistochemistry. Representative images are also included.

## 2. SCOPE AND APPLICABILITY

Inflammatory infiltrate in skeletal muscle is a hallmark of disease in MDC1A. This protocol describes an immunohistochemical approach to quantify the infiltration. The predominant inflammatory infiltrate is composed of macrophages and monocytes. CD11b (integrin  $\alpha M$ ) is a cell surface protein associated with macrophages and monocytes. This particular staining allows for accurate quantification of inflammatory cell infiltration.

## 3. CAUTIONS

Cautions with this particular staining are not unique to this staining alone.

- Good dissection and flash freezing techniques must be used in isolating skeletal muscle to ensure that the same muscle is evaluated across mice and that the architecture most closely mimics the natural tissue architecture.
- Embedding and cutting must be done carefully to ensure that the muscle is cut into cross-sectional and not longitudinal sections.

## 4. MATERIALS

- Precleaned treated glass slides (Surgipath 00302)
- Cover Glass Fisherbrand (12-545-M)
- Tissue Tek O.C.T. (Sakura #4583)
- Immedge Pen (Vector Laboratories H-4000)
- Phosphate Buffered Saline
- 5% and 1% Bovine Serum Albumin in PBS (Bovine Serum Albumin from Fisher BioReagents BP1605-100)
- 4% Paraformaldehyde (Paraformaldehyde from Fisher Scientific O4042-500)
- Sodium Hydroxide from LabChem Inc (LL24460-1)
- FITC rat anti-mouse CD11b Clone M1/70 (BD Pharmingen #553310)
- Vectashield with DAPI (Vector Laboratories H-1500)

Solutions:

5% BSA – add 0.5g of BSA to 10 mls of 1XPBS

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1% BSA – add 2 mls of 5% BSA to 8 mls 1XPBS  
4% Paraformaldehyde – Add 4g of paraformaldehyde to 100mls diH<sub>2</sub>O along with 1 drop NaOH. Let sit in 65°C water bath overnight until the PFA is in solution.

Equipment:

Fluorescent microscope

## **5. METHODS**

### **5.1 Sample collection and embedding**

Muscles which are of interest (e.g. TA, EDL, Triceps, Gastrocnemius, Diaphragm, etc) should be harvested by careful dissection. After dissection muscles should be flash-frozen in liquid nitrogen cooled isopentane. Samples can then be stored at -80°C until ready for use.

### **5.2 Embedding tissue and cryosectioning**

Tissue should be removed from -80°C storage and kept on dry ice before sectioning. Prior to mounting the tissue, it should be placed in the cryostat (at -20°C) and allowed to equilibrate at that temperature (about 10-20 minutes). The tissue should then be embedded in O.C.T. and mounted on the cryostat chuck. The muscle should be advanced to approximately the mid-belly section. Sections should be cut which are 8-10 μm thick and placed on clean, pre-coated slides. For best results with staining the slides should be used on the same day they are prepared, however, they can also be stored at -80°C.

### **5.3 CD11b staining**

This protocol will describe how to stain the CD11b positive cells green and the nuclei blue.

1. Perform all steps at room temperature in a light protected humidified chamber.
2. Outline the sections using an Immudedge pen. Be careful not to let the sections dry out.
3. Fix the sections using 4% paraformaldehyde for 3 minutes.
4. Wash slides with PBS three times at five minutes per wash.
5. Block the slides for one hour using 5% BSA.
6. Wash slides with 1% BSA three times at five minutes per wash.
7. Incubate sections with FITC rat anti-mouse for one hour.

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8. Wash slides with PBS three times at five minutes per wash.
9. Mount slides using Vectashield with DAPI avoiding air bubbles.

#### 5.4 Image acquisition

Capture images using a fluorescent microscope and camera (i.e. A Zeiss Axioskop 2 Plus fluorescent microscope, a Zeiss AxioCam HRc digital camera and Axiovision 4.1 software).

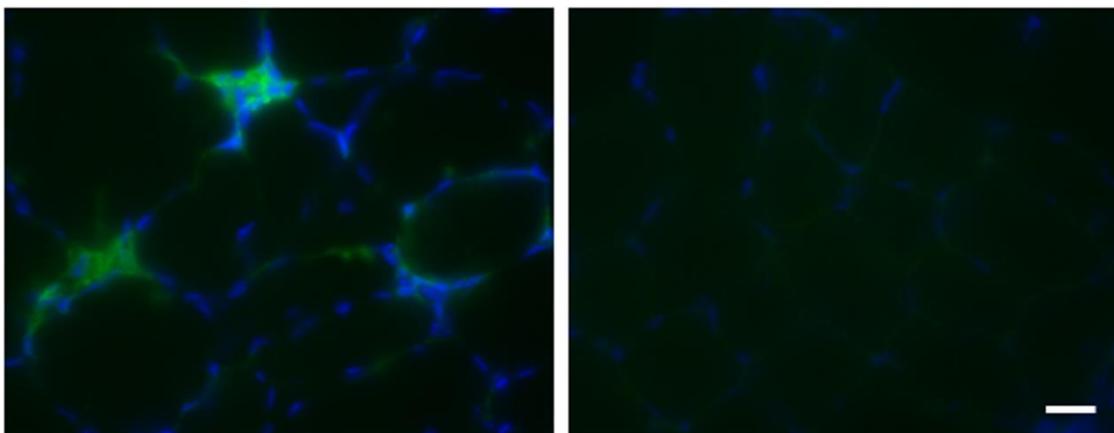
### 6. EVALUATION AND INTERPRETATION OF RESULTS

#### 6.1 Quantification of inflammatory infiltrate

Count all CD11b positive cells in twenty 400X fields. The number of CD11b cells per field is then determined and reported. Switching between the DAPI image and the CD11b image will facilitate this. Figure 1 in the Appendix shows a wild type section with no CD11b positive cells and an image from a  $dy^W/dy^W$  animal showing distinct areas of inflammatory infiltrate.

A statistical package such as GraphPad Prism can be used to determine significance between the experimental groups.

### 7. APPENDIX



**Figure 1. Inflammatory infiltrate in the  $dy^W/dy^W$  mouse.** Areas of inflammation stain green. Muscle fibers and non-inflammatory cells do not stain. Scale bar=20 $\mu$ m