

# Intact Short, Intermediate, and Long Skeletal Muscle Fibers Obtained by Enzymatic Dissociation of Six Hindlimb Muscles of Mice: Beyond Flexor Digitorum Brevis

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## Materials

| Name                                    | Company                              | Catalog Number | Comments                                        |
|-----------------------------------------|--------------------------------------|----------------|-------------------------------------------------|
| <b>Reagents</b>                         |                                      |                |                                                 |
| Absolute ethanol                        | Sigma Aldrich                        | 32221          |                                                 |
| Acetone                                 | Merck                                | 179124         |                                                 |
| Acrylamide                              | Gibco BRL                            | 15512-015      |                                                 |
| Ammonium persulfate                     | Panreac                              | 141138.1610    |                                                 |
| Anti myosin I antibody                  | Sigma Aldrich                        | M4276          | Primary antibody                                |
| Anti myosin II antibody                 | Sigma Aldrich                        | M8421          | Primary antibody                                |
| Anti myosin IIA antibody                | American Type Culture Collection     | SC-71          | Primary antibody. Derived from HB-277 hybridoma |
| Anti myosin IIB antibody                | Developmental Studies Hybridoma Bank | BF-F3-c        | Primary antibody                                |
| Bis-acrylamide                          | AMRESCO                              | 0172           |                                                 |
| Bovine serum albumin                    | Thermo Scientific                    | B14            |                                                 |
| Bradford reagent                        | Merck                                | 1.10306.0500   |                                                 |
| Bromophenol blue                        | Carlo Erba                           | 428658         |                                                 |
| Calcium carbonate                       | Merck                                | 102066         |                                                 |
| Calcium dichloride (CaCl <sub>2</sub> ) | Merck                                | 2389           |                                                 |
| Chloroform                              | Sigma Aldrich                        | 319988         |                                                 |
| Collagenase type 2                      | Worthington                          | CLS-2/LS004176 |                                                 |
| Consul-Mount                            | Thermo Scientific                    | 9990440        |                                                 |
| Coomassie Brilliant blue R 250          | Merck                                | 112553         |                                                 |
| Dimethyl sulfoxide (DMSO)               | Sigma Aldrich                        | D2650          |                                                 |
| Dithiothreitol (DTT)                    | AMRESCO                              | 0281           |                                                 |

|                                                 |                             |                        |                                                                                                                                                                              |
|-------------------------------------------------|-----------------------------|------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Edetic acid (EDTA)                              | AMRESCO                     | 0322                   |                                                                                                                                                                              |
| Eosin Y                                         | Sigma Aldrich               | E4009                  |                                                                                                                                                                              |
| Glycerol                                        | Panreac                     | 1423291211             |                                                                                                                                                                              |
| Glycine                                         | Panreac                     | 151340.1067            |                                                                                                                                                                              |
| Goat serum                                      | Sigma Aldrich               | G9023                  |                                                                                                                                                                              |
| Hematoxylin                                     | Thermo Scientific           | 6765015                |                                                                                                                                                                              |
| HEPES                                           | AMRESCO                     | 0511                   |                                                                                                                                                                              |
| Hoechst 33258                                   | Sigma Aldrich               | 861405                 |                                                                                                                                                                              |
| Imidazole                                       | AMRESCO                     | M136                   |                                                                                                                                                                              |
| Isopentane                                      | Sigma Aldrich               | M32631                 |                                                                                                                                                                              |
| Laminin                                         | Sigma Aldrich               | L2020                  |                                                                                                                                                                              |
| Mag-Fluo-4, AM                                  | Invitrogen                  | M14206                 | Prepared only in DMSO. Pluronic acid is not required and should not be used to avoid fiber deterioration.                                                                    |
| Mercaptoethanol                                 | Appllichem                  | A11080100              |                                                                                                                                                                              |
| Methanol                                        | Protokimica                 | MP10043                |                                                                                                                                                                              |
| Mice                                            | Several                     | Several                | For this manuscript, we only used C57BL/6 mice. However, some preliminary results have shown that the protocol works well for Swiss Webster mice of the same age and weight. |
| Mowiol 4-88                                     | Sigma Aldrich               | 81381                  |                                                                                                                                                                              |
| N,N,N',N'-tetramethylethane-1,2-diamine (TEMED) | Promega                     | V3161                  |                                                                                                                                                                              |
| N-benzyl-p-toluene sulphonamide (BTS)           | Tocris                      | 1870                   |                                                                                                                                                                              |
| Optimal cutting compound (OCT)                  | Thermo Scientific           | 6769006                |                                                                                                                                                                              |
| Secondary antibody                              | Thermo Scientific           | A-11001                | Goat anti-mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488                                                                                                 |
| Sodium dodecil sulfate                          | Panreac                     | 1323631209             |                                                                                                                                                                              |
| TRIS 0.5 M, pH 6.8                              | AMRESCO                     | J832                   |                                                                                                                                                                              |
| Tris(Hydroxymethyl)aminomethane                 | AMRESCO                     | M151                   |                                                                                                                                                                              |
| Triton X-100                                    | AMRESCO                     | M143                   |                                                                                                                                                                              |
| <b>Materials</b>                                |                             |                        |                                                                                                                                                                              |
| Dissection chamber                              | Custom-made                 |                        |                                                                                                                                                                              |
| Charged slides                                  | Erie Scientific             | 5951PLUS               |                                                                                                                                                                              |
| Experimental bath chamber                       | Warner Instruments          | RC-27NE2               | Narrow Bath Chamber with Field Stimulation, ensembled on a heated platform PH-6                                                                                              |
| Fine forceps                                    | World Precision Instruments | 500338, 500230         |                                                                                                                                                                              |
| Fine scissors                                   | World Precision Instruments | Vannas Scissors 501778 |                                                                                                                                                                              |
| Glass Pasteur pipettes                          | Several                     |                        | Fire-polished tips                                                                                                                                                           |
| Glass vials with cap                            | Several                     |                        | 2-3 mL volumen                                                                                                                                                               |
| Operating scissors                              | World Precision Instruments | 501223-G               |                                                                                                                                                                              |
| <b>Equipment</b>                                |                             |                        |                                                                                                                                                                              |
| Centrifuge                                      | Thermo Scientific           | SL 8R                  |                                                                                                                                                                              |
| Confocal microscope                             | Olympus                     | FV1000                 |                                                                                                                                                                              |
| Cryostat                                        | Leica                       | CM1850                 |                                                                                                                                                                              |
| Digital camera                                  | Zeiss                       | Erc 5s and Axio 305    | Axio 305, coupled to the Stemi 508 stereoscope, was used to take pictures during dissection; while Erc                                                                       |

|                                                                         |                               |                                                    |                                                                                                                                               |
|-------------------------------------------------------------------------|-------------------------------|----------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                         |                               |                                                    | 5s or Axio 208, coupled to the Axio Observer A1 microscope, were used to take images of the isolated fibers and the immunofluorescence assays |
| Digitizer                                                               | Molecular Devices             | 1550A Digidata                                     |                                                                                                                                               |
| Electrophoresis chamber                                                 | Bio Rad                       | Mini-Protean IV                                    |                                                                                                                                               |
| Inverted microscope coupled to fluorescence                             | Zeiss                         | Axio Observer A1                                   | Coupled to an appropriate light source, filters and objectives for fluorescence                                                               |
| Photomultiplier                                                         | Horiba                        | R928 tube, Hamamatsu, in a D104 photometer, Horiba | Coupled to the lateral port of the fluorescence microscope                                                                                    |
| Stereoscope                                                             | Zeiss                         | Stemi 508                                          |                                                                                                                                               |
| Stimulator                                                              | Grass Instruments             | S6                                                 |                                                                                                                                               |
| Water bath                                                              | Memmert                       | WNE-22                                             |                                                                                                                                               |
| Xilol                                                                   | Sigma Aldrich                 | 808691                                             |                                                                                                                                               |
| <b>Software</b>                                                         |                               |                                                    |                                                                                                                                               |
| Free software for electrophoreses analyses                              | University of Kentucky        | GelBandFitter v1.7                                 | <a href="http://www.gelbandfitter.org">http://www.gelbandfitter.org</a>                                                                       |
| Free software for image analysis and morphometry                        | National Institutes of Health | ImageJ v1.54                                       | <a href="https://imagej.nih.gov/ij/index.html">https://imagej.nih.gov/ij/index.html</a>                                                       |
| Licensed software for Ca <sup>2+</sup> signals acquisition and analyses | Molecular Devices             | pCLAMP v10.05                                      | <a href="https://www.moleculardevices.com">https://www.moleculardevices.com</a>                                                               |
| Licensed software for statistical analyses and graphing                 | OriginLab                     | OriginPro 2019                                     | <a href="https://www.originlab.com/">https://www.originlab.com/</a>                                                                           |