Miltenyi Biotec

Isolation and cultivation of satellite cells from mouse skeletal muscle

Contents

1. Description

1.1 Background information

The protocol has been developed to isolate high yields of viable satellite cells from mouse skeletal muscle tissue. Cells can be cultured or analyzed by flow cytometry afterwards or differentiated into mature myotubes.

1.2 Reagent and instrument requirements

- gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- Skeletal Muscle Dissociation Kit, mouse and rat (# 130-098-305)
- Satellite Cell Isolation Kit, mouse (# 130-104-268)
- MACS® SmartStrainers (70 µm) (# 130-098-462) to remove cell clumps.
- (Optional) Anti-Integrin α-7 MicroBeads, mouse (# 130-104-261)
- Gelatine
- CD34 antibodies, mouse conjugated to, e.g., FITC and Anti-Integrin α-7 antibodies, mouse conjugated to, e.g., PE.
- Culture medium: 40% Dulbecco's Modified Eagle Medium (DMEM), 40% HAM´s F10 medium, 20% fetal bovine serum (FBS), 2.5 ng/mL Human FGF-2 (e.g. # 130-093-839), 100 U/mL penicillin, and 100 U/mL streptomycin.
- MyoD (C-20) antibodies
- (Optional) Differentiation medium: 95% DMEM, 5% horse serum, 100 U/mL penicillin, and 100 U/mL streptomycin.

2. Protocols

2.1 Sample preparation

▲ For details on the use of the gentleMACS Dissociators, refer to the gentleMACS Dissociator user manuals.

1. Determine tissue weight.
2. Dissociate mouse skeletal muscle according to the protocol of the Skeletal Muscle Dissociation Kit, mouse and rat including post-dissociation wash steps.

2.2 Magnetic separation

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 70 µm nylon mesh (MACS SmartStrainers (70 µm), # 130-098-462) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

1. Isolate mouse satellite cells according to the protocol of the Satellite Cell Isolation Kit.
2. (Optional) To further increase the purity of isolated satellite cells, e.g., for direct molecular analysis, the Anti-Integrin α-7 MicroBeads, mouse can be used. For details refer to the respective data sheet.

2.3 Flow cytometric analysis

▲ For a detailed immunofluorescent staining protocol refer to the data sheet of the CD34 and Anti-Integrin α-7 antibodies.

1. Incubate cells with CD34 and Anti-Integrin α-7 antibodies.
2. Analyze cells by using a flow cytometer, e.g., the MACSQuant® Analyzer 10.

2.4 Cell culture

1. Coat of culture dish with 0.1% gelatine (overnight or 1 hour at 37 °C).
2. Plate 5×10^4 cells per cm^2 in culture medium and culture for six days. Change medium every two days.
3. After six days in culture a confluent grown cell layer can be seen. Stain cells for microscope analysis with MyoD (C-20) antibodies.
4. (Optional) Differentiation and fusion into mature myotubes can be performed after seven days in culture. Therefore, remove culture medium and add differentiation medium. Culture for three days to induce differentiation.

All gentleMACS Protocols are available at **www.miltenyibiotec.com**.

SP009401

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 877 591 1060
macs@miltenyibiotec.com

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

SP009401