PROFUSE-S1-Skeletal Muscle Cell Differentiation, Fusion and Maturation Supplement

Instructions for Use

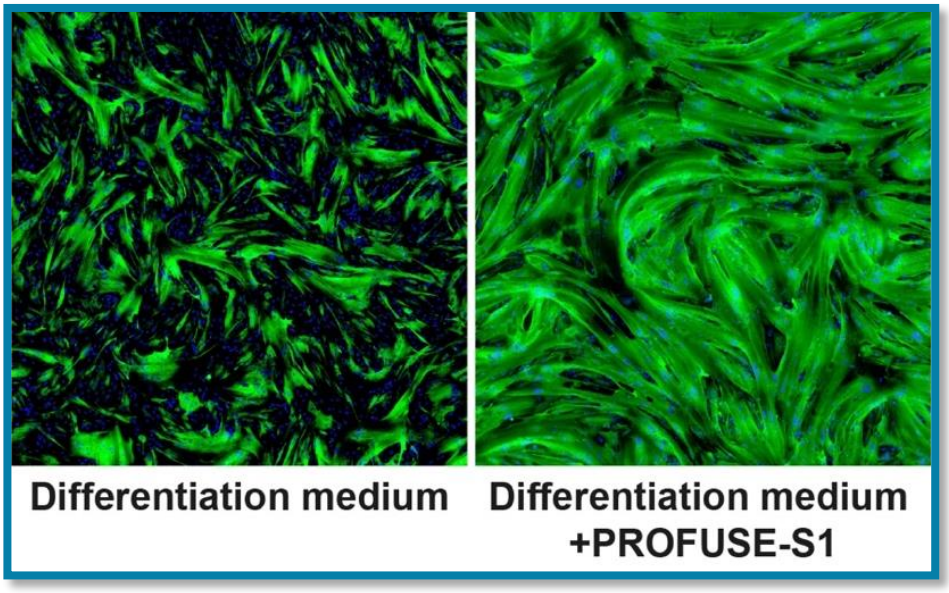

Figure 1: Bovine myoblasts - 48 hours after treatment with PROFUSE-S1 (alpha-sarcomeric actinin in green, nuclei in blue)

Introduction

Skeletal muscle has the ability to regenerate due to the presence of quiescent muscle progenitor cells (satellite cells) localized within the muscle fibers. Following injury, the satellite cells are activated and undergo asymmetric division to form myoblasts. Upon specific ques, myoblasts will further differentiate into myocytes and fuse with each other and to existing myotubes to regenerate the muscle. Similarly, myoblasts can undergo differentiation and fusion *in vitro* in culture. Myoblasts will begin to spontaneously form nascent myotubes in culture when the cells reach high confluency. Differentiation can be further promoted by treatment with differentiation medium, usually consisting of a reduced serum containing media. By adding PROFUSE-S1 into conventional differentiation media, the process of differentiation, fusion, and maturation of myotubes will proceed faster and more efficiently.

PROFUSE-S1 was validated on primary and immortalized myoblasts. As it was validated on primary myoblasts of various species (murine, bovine, ovine, porcine, avian, and piscine), PROFUSE-S1 is

predicted to work on human cells. Moreover, PROFUSE-S1 has been shown to be effective on immortalized myoblast cells including C2C12 cells, and is effective on fibroblasts that have been transdifferentiated by forced expression of MYOD1. PROFUSE-S1 is also effective on myoblasts used for the generation of 3D engineered bioartificial muscles using scaffolds and various hydrogels.

This protocol outlines the steps involved in differentiation of skeletal muscle myoblasts using the PROFUSE-S1 supplement in a 2D adherent setting.

Materials to be supplied by user

- Skeletal muscle myoblast cells
- Cell-specific growth media
- Cell-specific differentiation media
- DMSO - cell culture grade
- PBS-without Ca2+/Mg2+

Instructions for preparation of PROFUSE-S1 stock solution

Dissolve the entire contents of the supplied vial containing PROFUSE-S1 with 1.7 mL DMSO and vortex vigorously until dissolved, yielding 1000X stock solution.

Differentiation & Maturation Process

1. Culture skeletal muscle myoblasts under standard culturing conditions using suitable growth medium until the cell population reaches 70-80% confluency.
2. Remove growth medium, wash cells once with PBS and replace with the appropriate differentiation medium for the particular cells supplemented with PROFUSE-S1 (dilute stock solution 1:1000, instruction for preparation of stock solution is described above).
3. Replace with fresh differentiation medium after 24 hours, and every other day until myotubes are observed throughout the culture **(*do not reapply PROFUSE-S1**). The resulting differentiated cultures can be observed to contain multinucleated (containing more than 3 nuclei) myotubes. Time to completion of process will vary depending on the species of origin of the cells and the cell type (primary vs. immortalized cell line).
4. Expected time to endpoint of differentiation following application of PROFUSE-S1 to primary myoblasts are listed in the table below (results may vary based on quality of cells and technique)

Species	Time to completion
Murine	24 hours
Avian	48 hours
Bovine	72 hours
Ovine	36 hours
Porcine	72 hours

*Application note

PROFUSE-S1 should only be applied a ***single time*** to the cells, and not replenished upon media changes.

Alternatively, cells can be subjected to a 24-hour pretreatment with PROFUSE-S1 (1:1000) in growth media before being transitioned to differentiation media.

General comments:

1. PROFUSE-S1 synchronizes the differentiation process, and will result in extremely high efficiency rates of differentiation and fusion. Endpoints will vary by species.
2. To prevent spontaneous differentiation in growth media, it is essential to passage cells at a moderate confluency (60-70%). This approach helps maintain the undifferentiated state of the cells and optimizes their proliferation.
3. Mature myotubes grown in a 2D adherent culture are highly susceptible to detachment during handling. Therefore, the cultures should be manipulated with utmost care, and vigorous pipetting should be avoided.
4. The above protocol can be adapted for multiple species. PROFUSE-S1 is expected to work on human iPSC/ESC derived myoblasts.

Characterization of Cells

Following the induction of differentiation, myotubes can be fixed with paraformaldehyde and immunostained for positive expression of Myosin Heavy Chain or other sarcomeric proteins. Fusion indexes can be generated by calculating the number of total nuclei in a given field localized within multinucleated myotubes.