cyagen | OriCell

User Manual

OriCell™ Myotube Differentiation Medium For Muscle Cells

Catalog No. GUXMC-90401

We help you discover life

Introduction

OriCell™ Myotube Differentiation Medium For Muscle Cells, meticulously developed by our R&D team, is designed for the differentiation of muscle cells into myotubes. It provides a complete system, including an optimized basal medium, essential induction supplements and staining solution.

This product is effective for the myogenic induction of various muscle cells. Extensive cell culture data have demonstrated that it supports the stable and efficient differentiation of muscle cells (e.g., C2C12 cell line and primary skeletal muscle cells) into myotubes.

Note: **This product is intended for research use only and is not for diagnostic, therapeutic, clinical, household, or any other applications.**

When citing our products in academic publications, please use the following format: "OriCell™ [Product Name] + [Catalog Number], from Cyagen Biosciences."

Product Information

Components	Catalog Number	Volume	Storage
OriCell™ Basal Medium For Cell Culture	BHDM-03011	98 mL	4 °C
OriCell™ Supplements For Myotube Differentiation	GUXMC-04401	2 mL	-20 °C
OriCell™ Giemsa Solution	GMSA-10001	10 mL	RT

*Note: RT = Room Temperature (15-30 °C).

QC

- Pass the detection of bacteria, fungi, mycoplasma, and endotoxins.
- Pass the detection of osmotic pressure and pH.
- Pass the detection of product quality.

Please refer to "COA" for details.

General Handling Principles

1. Maintain strict aseptic technique. Ensure complete sterility throughout all procedures, particularly within the laminar flow hood and incubator.
2. Follow standardized protocols. Adhere strictly to the product manual instructions. Implement rigorous control over experimental variables and include appropriate parallel controls.
3. Ensure proper storage and use. Store all components according to specified conditions and use them promptly to ensure optimal performance.
4. Aliquot for long-term storage. If the entire volume will not be used up immediately, prepare the medium in batches according to the specified component volume ratios and store in aliquots.

Product Stability and Storage Conditions

1. All components must be stored away from light.
2. The basal medium and staining solution have a shelf life of 1 year and should be stored at 4 °C and room temperature (RT) respectively. The supplement has a shelf life of 2 years and should be stored at -20 °C.
3. Once prepared, the medium has a shelf life of 1 month when stored at 4 °C. The shelf life may be extended up to a maximum of 45 days, provided that culture conditions remain stable, the container is properly sealed, and repeated temperature fluctuations are avoided.
4. Use all components before the expiration dates. Expired components may severely compromise culture performance.

Preparation of Complete Medium

Materials Required

- OriCell™ Myotube Differentiation Medium For Muscle Cells (Cat. No.: GUXMC-90401)
- Clean, sterile, and stable quality disposable consumables (pipettes, pipette tips, centrifuge tubes, etc.)
- Clean sealing film
- Aluminum foil and other light-avoiding materials

Steps

1. At least 6 hours before preparation, place the OriCell™ Supplements For Myotube Differentiation (Cat. No.: GUXMC-04401) in a refrigerator at 4 °C to allow it to thaw completely.
2. Carefully wipe the outer packaging of all components with 75% ethanol. Open the package

inside a clean bench (laminar flow hood).

3. Add all of the supplements (Cat. No.: GUXMC-04401) to OriCell™ Basal Medium (Cat. No.: BHDM-03011).
4. Rinse each bottle and tube with a small volume of basal medium, then transfer the washings back to the basal medium bottle to maximize recovery.
5. Tighten the cap of the basal medium bottle and swirl the bottle gently to mix thoroughly.
6. Seal the bottle with Parafilm, wrap it in aluminum foil to protect from light, and label it with the product name, preparation date, and other relevant information.

Special Notes:

1) If the medium will not be used up immediately, we recommend preparing in batches. Please prepare the required amount according to the ratio of each component in the kit. Any remaining components must be stored according to their respective storage conditions and should not be subjected to multiple freeze-thaw cycles.

2) The prepared medium should be aliquoted into small portions to avoid repeated freeze-thaw cycles.

3) Please choose whether to add antibiotics (e.g., Penicillin/Streptomycin) according to your own needs. If required, it should be prepared or purchased separately.

4) All components in the OriCell™ Myotube Differentiation Medium For Muscle Cells are strictly aseptically controlled. Under normal circumstances, we do not recommend sterilizing it again. However, if there is a risk of contamination during the preparation process, the complete medium can be filtered and sterilized.

Procedure for Inducing Differentiation

Materials Required

- OriCell™ Myotube Differentiation Medium For Muscle Cells (Cat. No.: GUXMC-90401)
- OriCell™ C2C12 Mouse Myoblast Cell Line (Cat. No.: M7-0101)
- OriCell™ Complete Medium For C2C12 Cell Line (Cat. No.: CMM7-0101)
- OriCell™ 0.1% Gelatin Solution (Cat. No.: GLT-11301)
- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)

Steps

Note:

1) This protocol uses a 6-well plate as an example. Please select an appropriate culture vessel based on your specific needs.

2) To prevent cell detachment (floating), it is recommended to coat the culture vessel with 0.1% gelatin.

3) Pre-warm the induction medium to 37 °C before use.

4) This protocol uses the C2C12 cell line as an example.

1. Add 1 mL of 0.1% gelatin to each well of the 6-well plate. Gently swirl the plate to ensure the solution covers the bottom surface evenly.
2. Place the gelatin-coated plate on an ultraclean bench (laminar flow hood) or a CO_2 incubator for at least 30 minutes.
3. After 30 minutes, aspirate the gelatin solution. The plate can then be used immediately for cell seeding or air-dried before use.
4. Seed the C2C12 cells (Cat. No.: M7-0101) at a density of 2×10^4 cells/cm^2 and add 2 mL of regular complete medium (Cat. No.: CMM7-0101) to each well.

5. Incubate the cells at 37 °C, 5% CO_2, and saturated humidity.
6. When the cells reach 90% confluence, carefully aspirate the complete medium and add 2 mL of myotube differentiation medium (Cat. No.: GUXMC-90401) to each well.
7. Replace the medium with fresh myotube differentiation medium every 2-3 days.
8. After 3–7 days of induction, perform Giemsa staining, depending on cell morphology and growth status.

Giemsa Staining Analysis

Materials Required

- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)
- 4% Paraformaldehyde or 10% Formalin
- OriCell™ Giemsa Solution (Cat. No.: GMSA-10001)

Steps

Note:

(1) Handle all procedures gently to prevent detachment of cells.

(2) Ensure the presence of myotubes before staining.

(3) If staining is insufficient, the incubation time may be extended as appropriate.

1. After the induction process is complete, aspirate the myotube differentiation medium from the 6-well plate and wash each well gently 2–3 times with 1× PBS.
2. Add 2 mL of 4% paraformaldehyde (PFA) solution or 10% formalin solution to each well and fix the cells at room temperature for 30 minutes.
3. Aspirate the fixative and gently wash 2–3 times with 1× PBS to ensure complete removal.
4. Prepare the Giemsa working solution by diluting OriCell™ Giemsa Solution (Cat. No.: GMSA-10001)

in buffer solution at a ratio ranging from 1:5 to 1:10.

5. Add 2 mL of Giemsa working solution to each well and incubate for 30 minutes at room temperature.
6. Aspirate the Giemsa staining solution and rinse the wells 2–3 times with 1× PBS to ensure thorough removal of excess stain.
7. Add 2 mL of 1× PBS to each well and observe the staining results under a microscope.
8. Seal the plate with Parafilm and protect from light. The stained plate can be stored at 4 °C for up to 2 weeks.

Giemsa Staining Examples

Giemsa Staining Result of OriCell™ C2C12 Mouse Myoblast Cell Line (Cat. No.: M7-0101)

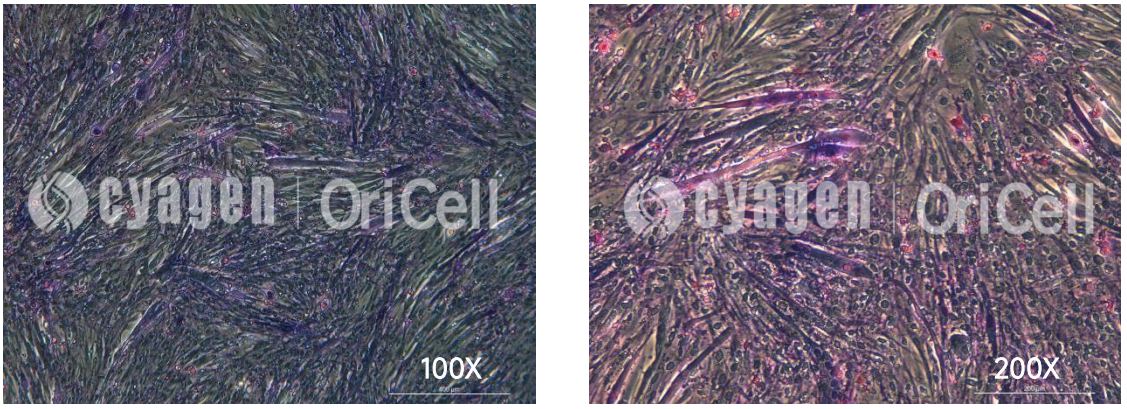


Figure 1. Staining results of cells (seeding density: 1 x 10^5) after induction.

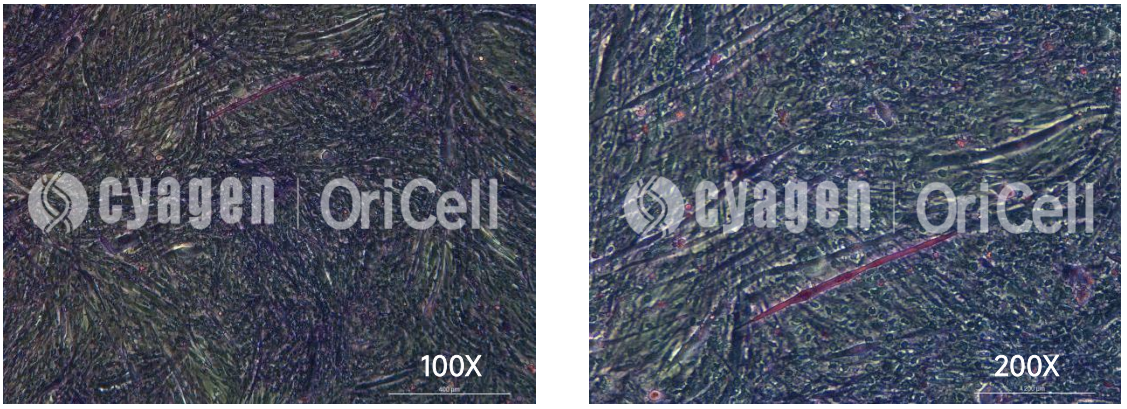


Figure 2. Staining results of cells (seeding density: 2 x 10^5) after induction.

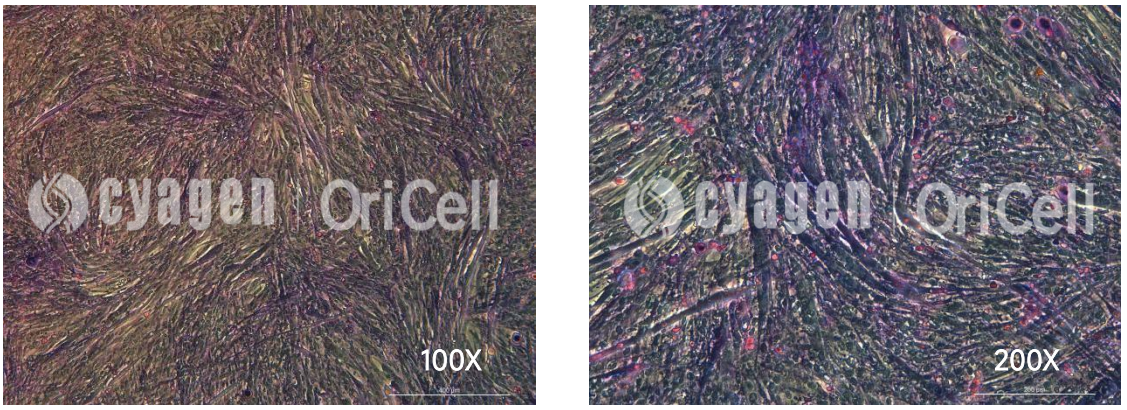
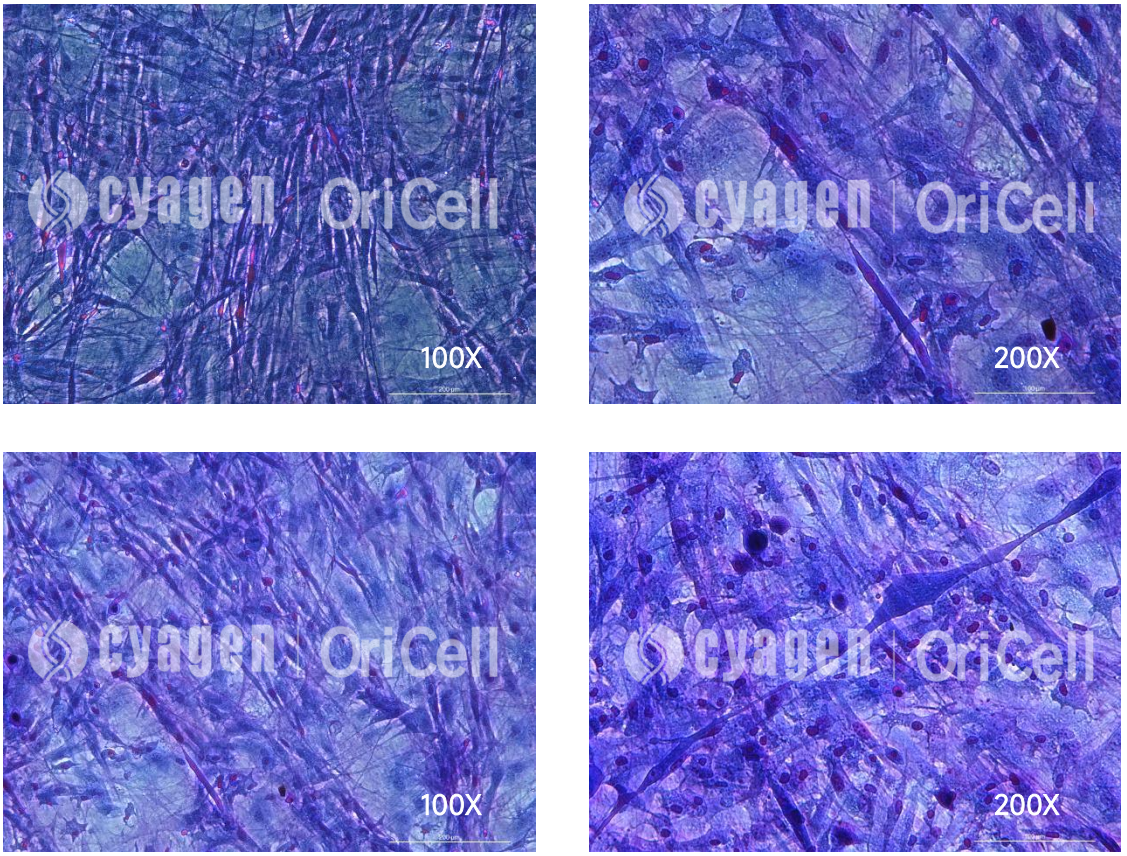


Figure 3. Staining results of cells (seeding density: 3 x 10^5) after induction.

Giemsa Staining Result of OriCell™ C57BL/6 Mouse Skeletal Muscle Cells (Cat. No.: MUBSM-02081)



Cyagen Biosciences (Suzhou) Inc. reserves all rights to the technical documents of OriCell™ cell culture products. Without the written permission of Cyagen Biosciences (Suzhou) Inc. any part of this document shall not be adapted or reprinted for other commercial purposes.